THE LIVER´S INFLUENCE ON IMMUNE CELL FUNCTION AND ITS CONSEQUENCE FOR LIVER DISEASE

February 13-14, 2025

Symposium

MUNICH, GERMANY

7 CME CREDITS

7 credit hours (CME) have been awarded by the European Union of Medical Specialists (UEMS).

PREFACE

Dear colleagues and friends,

it is my great pleasure to invite you to the Symposium “The Liver’s Influence on Immune Cell Function and its Consequence for Liver Desease” organized by the Falk Foundation e.V. The Symposium will take place from Thursday 13th to Friday 14th of February 2025 at the TUM University Hospital München Rechts der Isar and precede the annual meeting of the German Association for the Study of Liver Disease in 2025 in Munich.

This Symposium will address the important topic of local regulation of immune responses in the liver. Internationally known experts in the field will address key questions in liver immunology, macrophage biology, metabolic liver disease, and immune therapy strategies within the context of spatial biology in the liver. Given the important role of the liver as an immunological organ and the impact of ill-controlled immunity on the development of liver disease, this Symposium offers a unique opportunity for scientists and clinicians to learn, network, and contribute to our shared scientific knowledge on Liver Immunology.

I am very grateful to all the speakers who, with their scientific contributions, will make this meeting a success and to the Falk Foundation e.V. for their relentless effort in supporting science to improve our understanding of liver diseases.

The local scientific organizing team and I are looking forward to seeing you in Munich!

Sincerely,

Percy A. Knolle

THE LIVER´S INFLUENCE ON IMMUNE CELL FUNCTION AND ITS CONSEQUENCE FOR LIVER DISEASE

February 13-14, 2025

Scientific Organization:
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Congress Venue:
Klinikum rechts der Isar
Technische Universität München - MRI
Hörsaal B
Ismaninger Straße 22
81675 Munich
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For admission to scientific events your name badge should be clearly visible.

Accompanying persons are not permitted during the conference at any time.

Call for Posters:
An ePoster session will be held.
For details see page 11.

Start of Registration:
Thursday, February 13, 2025
12:00 - 18:00 h
at the congress office

Publication Date of the Final Program:
February 2025

The final program will be available on the website www.falkfoundation.org one week before the start of the symposium.

Thursday, February 13, 2025

12:00	Registration and lunch with ePoster session
13:10	Welcome *Percy A. Knolle, Munich*

SESSION I

Influence of the Liver on Immune Cell Function

Chairs: *Christian Kurts, Bonn; Christoph Neumann-Haefelin, Cologne*

13:15	Local regulation of CD8 T cell function in the liver *Laura Pallett, London*
13:40	Dynamics and function of memory CD8 T cells in the liver *Maike Hofmann, Freiburg*
14:05	Restoration of hepatic CD8 T cell immunity in chronic infection *Dirk Wohlleber, Munich*
14:30	Role of innate-like T cells in the liver for local immunity *Katrin Böttcher, Munich*
14:55	Impact of CD4 T cells on the regulation of liver immunity *Ye Htun Oo, Birmingham*
15:20	**Coffee break with ePoster session**

Thursday, February 13, 2025

SESSION II

MAFLD and Immune Cell Function

Chairs: *Tobias Böttler, Freiburg; Mathias Heikenwälder, Tübingen*

16:00	Immune cells and platelets in liver immunity and MASH *Mathias Heikenwälder, Tübingen*
16:25	Immunity, fibrosis and metabolic liver disease *Scott L. Friedman, New York*
16:50	Role of liver macrophages in metabolic liver disease *Frank Tacke, Berlin*
17:15	CD8 T cell auto-aggression and metabolic liver disease *Michael Dudek, Munich*
17:40	Immune cell crosstalk in the kidney in health and disease *Christian Kurts, Bonn*
18:05	**Networking and ePoster session with light refreshments**

Friday, February 14, 2025

SESSION III

Unique Functions of Liver Macrophages

Chairs: *Jan Böttcher, Munich; Johannes Herkel, Hamburg*

09:00	Unique functions of liver macrophages *Thomas Fabre, Cambridge*
09:20	Hepatic fibrosis and liver macrophage function *Moritz Peiseler, Berlin*
09:40	Maternal obesity and its long-lasting effects on liver macrophages *Elvira Mass, Bonn*
10:00	Spatial biology of liver macrophages and their function *Patrick Bertolino, Sydney*
10:20	Cell death and liver macrophage function *Lidia Bosurgi, Hamburg*
10:40	**Coffee break with ePoster session**

Friday, February 14, 2025

SESSION IV

Immune Therapy and the Liver

Chairs: *Ursula Ehmer, Munich; Christian Lange, Munich*

11:00	Immune therapy of liver cancer *Tim F. Greten, Bethesda*
11:20	Therapeutic vaccination against chronic hepatitis B *Ulrike Protzer, Munich*
11:40	Liver Transplantation and immune therapy *Alberto Sánchez-Fueyo, London*
12:00	Therapy of autoimmune liver disease *Christoph Schramm, Hamburg*
12:20	Novel imaging technologies for future guidance of decision-making *Ron Heeren, Maastricht*
12:40	**Lunch with ePoster session**
13:15	**Opening of the annual meeting of the GASL**

LIST OF SPEAKERS, MODERATORS AND SCIENTIFIC ORGANIZERS

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REGISTRATION

You can register for the event via our homepage:
www.falkfoundation.org
Registration is only possible online.

You will receive an automatic confirmation of registration by e-mail. Please transfer the congress fee to the bank account listed in the e-mail within two weeks.

CONGRESS FEES

Scientific Program of Symposium	EUR 150
Students (copy of student ID required)	EUR 75

The congress fees include:
- Refreshments during coffee breaks
- Lunch on Thursday, February 13 and Friday, February 14, 2025
- Snacks during scientific discussion on Thursday, February 13, 2025
- A copy of the final program

CONGRESS OFFICE AND REGISTRATION

Opening Hours:

Thursday, February 13, 2025	12:00 - 18:00 h
Friday, February 14, 2025	8:00 – 12:30 h

The Falk Foundation will take pictures during the meeting. Additionally, parts of the meeting might be recorded. By participating all attendees consent and agree with the recording and the photo shoots.

POSTER SESSION

ePosters will be exhibited on our ePoster screens on February 13-14, 2025. The authors will be in attendance during coffee and lunch breaks on both days.

ARRIVAL

Klinikum rechts der Isar
Technische Universität München - MRI

Hörsaal B
Ismaninger Straße 22
81675 Munich
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By Train & Tram

From Munich Central Station, take the underground line U4 towards Arabellapark or U5 towards Neuperlach Süd. From both underground lines it is four stops to Max-Weber-Platz. From here, follow the signs for 'MRI' to Ismaninger Straße.

From Ostbahnhof, take the U5 underground line in the direction of Laimer Platz and get off at Max-Weber-Platz. From here, follow the signs for 'MRI' to Ismaninger Straße.

By Plane

Munich Airport is located approx. 30 kilometres outside the city centre. From the airport, take the S-Bahn line S8 to the München Ostbahnhof stop. Change there to the underground line U5 in the direction of Laimer Platz and travel one stop to Max-Weber-Platz. From here, follow the signs for 'MRI' to Ismaninger Straße.

CONFLICTS OF INTEREST

Members of the scientific committee declare the following potential conflicts of interest:
Percy A. Knolle: no potential conflict of interest to report

POSTER ABSTRACTS

1. 3D liver micro-organoid model to study metabolic dysfunction-associated steatotic liver disease (MASLD)
   R. Aspera-Werz, M. Hammour, Y. Xin, G. Chen, B. Kaufmann, D. Hartmann, A. Nussler (Tübingen, DE)
2. Profibrogenic crosstalk between cholangiocytes and mucosal-associated invariant T (MAIT) cells in primary sclerosing cholangitis
   A. Azad, F. Gutierrez, M. Baez-Faria, A. Wixom, K. Lazaridis, G. Gores (Rochester, US)
3. Secretome from hepatic innate immune cells in activating stellate cells
   E. Bahrami (Biberach, DE)
4. A case with seronegative autoimmune hepatitis: Too often missed or delayed in clinical practice
   M. Basaranoglu, G. Basaranoglu, Z. Demirkok (Istanbul, TR)
5. Hepatocyte oxidative stress and inflammatory responses enhance osteoclast function in a human 3D in vitro liver-bone co-culture system
   G. Chen, Y. Xin, M. Hammour, A. Nuessler, R. Aspera-Werz (Tübingen, DE)
6. Mass spectrometry-based untargeted metabolomics study of non-obese individuals with non-alcoholic fatty liver disease
   M. Demirel, F. Koktasoglu, E. Ozkan, H. Dulun, A. Gul, R. Sharifov, U. Sarikaya, M. Basaranoglu, S. Selek (Istanbul, TR)
7. The role of inhibitor of differentiation proteins in hepatocellular carcinoma
   H. Ehnis, C. Hellerbrand (Erlangen, DE)
8. Bone morphogenetic protein 13 exhibits prometastatic effects on melanoma cells
   V. Freutsmiedl, T. Seitz, C. Hellerbrand (Erlangen, DE)
9. Autoimmune hepatopathies: What are the epidemiological and evolutionary particularities?
   H. Gdoura, L. Mnif, N. Tahri (Sfax, TN)
10. Liver sinusoidal endothelial cells maintain T cell tolerance induction by in liver fibrosis
    C. Gottwick, P. Averhoff, D. Krzikalla, R. Digigow, S. Fleischer, A. Lohse, A. Carambia, J. Herkel (Hamburg, DE)
11. Platelet count to mean platelet volume ratio as a survival predictor in patients with hepatocellular carcinoma
    M. Hamzaoui, I. Keskes, A. Ben Mohamed, M. Yaakoubi, G. Gharbi, M. Mahmoudi, A. Khsiba, M. Medhioub (Nabeul, TN)
12. The HALP score: A new biomarker for prognostic stratification in hepatocellular carcinoma
    M. Hamzaoui, I. Keskes, A. Ben Mohamed, M. Yaakoubi, G. Gharbi, M. Mahmoudi, A. Khsiba, M. Medhioub (Nabeul, TN)
13. The ITA.LI.CA score: A new predictor of overall survival in patients with hepatocellular carcinoma
    M. Hamzaoui, I. Keskes, M. Yaakoubi, G. Gharbi, A. Ben Mohamed, M. Mahmoudi, A. Khsiba, M. Medhioub (Nabeul, TN)
14. Primary biliary cholangitis: Predictive factors for decompensation
    G. Hela, L. Mnif, N. Tahri (Sfax, TN)

15. The role of stanniocalcin 2 in hepatocellular carcinoma
P. Hoefer, C. Hellerbrand (Erlangen, DE)

16. Adipocyte secreted factors induce tumorigenic and prometastatic behaviour of hepatocellular carcinoma cells in vitro
T. Itzenhaeuser, J. Sommer, C. Hellerbrand (Erlangen, DE)

17. Immunoregulation and MDSC-mediated T cell paralysis in hepatocellular carcinoma following rVSV-NDV therapy
M. Jimenez, B. Hoechst, J. Altomonte, P. Knolle, S. Donakonda, J. Marek, L. Hanesch, S. Glauss (Munich, DE)

18. Expression and function of G protein-coupled receptor 37 in hepatic fibrosis
S. Jochem, C. Hellerbrand (Erlangen, DE)

19. Neutrophil-to-lymphocyte ratio (NLR) and systemic inflammation index (SII): Predictive inflammatory markers of advanced hepatocellular carcinoma
I. Keskes, A. Ben Mohamed, G. Gharbi, M. Yaakoubi, M. Mahmoudi, A. Khsiba, M. Medhioub, M. Hamzaoui (Nabeul, TN)

20. The Metroticket 2.0 score: A powerful predictor of progression-free survival in patients with hepatocellular carcinoma
I. Keskes, A. Ben Mohamed, G. Gharbi, M. Yaakoubi, M. Mahmoudi, A. Khsiba, M. Medhioub (Nabeul, TN)

21. Dietary modification with oral probiotics influences regulation of immune cells in MASLD
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22. Serum sterols profiles reflect altered cholesterol homeostasis in cirrhotic patients with PBC and correlate with response to ursodeoxycholic acid
M. Krawczyk, W. Smyk, B. Kruk, T. Rezen, M. Moskon, E. Wunsch, F. Lammert, D. Lutjohann (Essen, Hannover, Bonn, DE; Gdansk, Warsaw, Szczecin, PL; Ljubljana, SI)

23. Lysine-specific demethylase 1 (LSD1) modulates hepatic lipid metabolism in metabolic dysfunction-associated fatty liver disease
H. Makwana, J. Wang, X. Zhao, H. Eischeid-Scholz, V. Kondylis, M. Escobar, R. Buettner, M. Odenthal (Cologne, Düsseldorf, DE)

24. Predictive factors for degeneration in compensated post-viral cirrhosis
M. Medhioub, A. Ben Mohamed, M. Yacoubi, M. Mahmoudi, G. Gharbi, A. Khsiba, L. Hamzaoui (Nabeul, TN)

25. Important roles of HBe and HBs antigens in the evasion of endogenous innate immune responses in hepatitis B virus infection
L. Muungani (Essen, DE)

26. Bile acid receptor Tgr5 as a modulator of macrophage immunometabolism during infection
M. Reich, T. Franz, P. Philippski, S. Kahlfuss, V. Keitel (Magdeburg, DE)

27. Identification and validation of gene expression pattern in a cell culture model of Wilson's disease
M. Sasula, A. Held, H. Schmidt, R. Broering (Essen, DE)

28. Schistosoma mansoni egg-derived antigens stimulate metabolic activity in the liver and colon by engaging insulin-like growth factor-1 receptor signaling pathways
F. Schmidt, V. Von Buelow, F. Stettler, V. Wirth, R. Soelter, G. Schramm, C. Grevelding, M. Roderfeld, E. Roeb (Giessen, Borstel, DE)

29. Is gluten-free diet a guarantee for healthy diet and optimal weight?
M. Selak, E. Kolak, R. Despot (Split, HR)

30. Hobit+ liver resident lymphocytes in MASH-HCC
P. Shen, C. Fan, S. Kan, A. Kadianaki, T. Bieler, S. Prokosch, U. Rothermel, F. Mueller, A. Eck, A. Kallies, M. Heikenwaelder (Heidelberg, Tübingen, DE; Melbourne, AU)

31. Liver macrophages release IL-6 and TNF-alpha upon ADAM17 and EGF-receptor activation by augmenter of liver regeneration (ALR)
S. Sigl, T. Weiss, C. Voigt, M. Kubitza, L. Buerger, M. Melter (Regensburg, DE)

32. Results of combined prophylaxis in preventing hepatitis B virus recurrence in liver transplant recipients – Insights of a single-center experience
A. Singeap, I. Girleanu, L. Huiban, C. Muzica, C. Stanciu, M. Blaj, A. Trofin, C. Lupascu, A. Trifan (Iasi, RO)

33. Factors secreted by adipocytes induce the tumorigenic and prometastatic properties of melanoma cells
B. Starke, J. Sommer, C. Hellerbrand (Erlangen, DE)

34. The complex interplay of cytokines, immune cells, and microbial endotoxin in experimental model of hepatic injury signifies liver's role in immune regulation
A. Sydorchuk, I. Hryhorchuk, R. Sydorchuk, L. Sydorchuk, R. Knut, I. Sydorchuk, I. Sydorchuk, I. Plehutsa (Neu-Ulm, Siegen, DE; Chernivtsi, Storozhynets, UA)

35. Genetically determined linkage of metabolism, hepatic functions, adipokines and CD4+ cells in MASLD
L. Sydorchuk, I. Hryhorchuk, A. Sydorchuk, R. Sydorchuk, R. Knut, I. Sydorchuk, O. Plehutsa, I. Sydorchuk, I. Zabolotna (Chernivtsi, Kyiv, UA; Neu-Ulm, Siegen, DE)

36. In vivo study of the natural killer T-cells in diet-related metabolic dysfunction associated liver disease
R. Sydorchuk, I. Hryhorchuk, L. Sydorchuk, A. Sydorchuk, I. Plehutsa, I. Sydorchuk, I. Sydorchuk (Chernivtsi, Storozhynets, UA; Neu-Ulm, Siegen, DE)

37. Hepatic and extrahepatic complications in autoimmune hepatitis (case report)
E. Tcholadze, M. Kanashvili, T. Sokolova, M. Tsimakuridze, K. Tsanava, K. Khatiashvili (Tbilisi, GE)

38. Inverse correlation between Th2-associated cytokines and hepatic egg burden in Schistosoma mansoni-infected hamsters
V. Von Buelow, L. Russ, F. Stettler, G. Schramm, F. Falcone, C. Grevelding, M. Roderfeld, E. Roeb (Giessen, Borstel, DE)

39. Alcohol-induced fibrosis in a long-term 3D human liver model
Y. Xin, G. Chen, M. Hammour, R. Aspera-Werz, A. Nuessler (Tübingen, DE)

40. T-cell receptor analysis of circulating T cells reveals non-epitope-driven clonal expansion in metabolic associated steatotic liver disease
X. Zhao, S. Aschenbroich, X. Yu, S. Lyu, U. Koitzsch, N. Sereda, K. Rheinwalt, A. Plamper, N. Fang, R. Schierwagen, J. Trebicka, U. Drebber, M. Odenthal, M. Brol (Cologne, Münster, DE)

FULL CONTENT OF POSTER ABSTRACTS

Poster Numbers 1 – 40

1. 3D liver micro-organoid model to study metabolic dysfunction-associated steatotic liver disease (MASLD)

Romina Aspera-Werz (Tübingen, DE), Majd Hammour (Tübingen, DE), Yuxuan Xin (Tübingen, DE), Guanqiao Chen (Tübingen, DE), Benedikt Kaufmann (Tübingen, DE), Daniel Hartmann (Tübingen, DE), Andreas Nuessler (Tübingen, DE)

Introduction: Metabolic dysfunction-associated steatotic liver disease (MASLD) represents a growing global health concern, particularly as it progresses toward more severe conditions like metabolic-associated steatohepatitis (MASH), liver fibrosis, and cirrhosis. Current in vivo models for studying MASLD often rely on animal systems, which present limitations in replicating human metabolic pathways and responses. To overcome these challenges, we developed a novel 3D liver micro-organoid model that mimics MASLD pathophysiology, offering a more predictive and human-relevant platform for studying disease mechanisms.

Methods: This model utilizes co-cultured HepaRG hepatocytes, LX-2 stellate cells, and HUVEC endothelial cells, which self-assemble into spheroids over two weeks. MASLD was induced by treating the spheroids with palmitic and oleic acids to simulate steatosis. Disease induction was confirmed through Bodipy staining, which visualized lipid accumulation, as well as by the upregulation of perilipin-2 and apolipoprotein C3 – both key markers of lipid metabolism. The model's metabolic activity and gene expression were further characterized by RT-PCR, demonstrating a significant reduction in Cytochrome P450 (CYP) enzymes, including CYP1A2, CYP3A4, and CYP2C9. Those results reflect impaired drug metabolism similar to that seen in human MASLD patients.

Results: Additionally, a notable downregulation in the expression levels of key enzymes involved in the metabolism and activation of vitamin D, specifically CYP27A1 and CYP2R1, was observed. To further substantiate these findings, RNA sequencing data from NCBI was analyzed, comparing liver samples from patients with MAFLD to those of healthy individuals. The differential expression analysis revealed downregulation of CYP27A1, and a decrease in vitamin D receptor expression consistent with the experimental in vitro results.

Discussion/Conclusion: By providing a human-specific in vitro model of MASLD, this liver micro-organoid system represents a promising tool to investigate disease progression and better understand the molecular underpinnings of MASLD. It serves as a pre-clinical platform to test potential therapeutic interventions to slow or reverse liver damage in patients with steatotic liver disease.

2. Profibrogenic crosstalk between cholangiocytes and mucosal-associated invariant T (MAIT) cells in primary sclerosing cholangitis

Adiba Azad (Rochester, US), Florencia Gutierrez (Rochester, US), Michelle Baez-Faria (Rochester, US), Alex Wixom (Rochester, US), Konstantinos Lazaridis (Rochester, US), Gregory Gores (Rochester, US)

Introduction: Primary sclerosing cholangitis (PSC) is an immune mediated liver disease that causes chronic inflammation and fibrosis of the extra and intrahepatic bile ducts. There is a large on met need for effective treatment for PSC, and at present liver transplan-

tation remains the only effective intervention for this disease. The primary cell impacted in PSC are cholangiocytes, which are bile duct epithelial cells. There is increasing evidence that this regulation of the immune system, in particular T lymphocytes existing patients with PSC. The IL-17 family of cytokines includes members IL-17A to IL-17F. The prototypical member is IL-17A which has been implicated in promoting liver fibrosis. However, IL-17A directed therapies have been largely unsuccessful in treating autoimmune diseases of the GI tract. IL-17C is a unique member of the family that has functions in innate immunity and epithelial cell defense. It has not been studied in PSC. Mucosal associated in variant T (MAIT) are a subtype of unconventional T-cell that are abundant in the human liver, and have been previously implicated in liver fibrosis. The premise of this study is to investigate a novel crosstalk between cholangiocytes and MAIT cells mediated by IL-17C and the consequent impact on liver fibrosis in PSC.

Methods: Specimens used in this project are human donor and PSC patient peripheral blood mononuclear cells (PBMC) to isolate and study MAIT cells, bile duct brushings obtained from PSC patients to profile cells in the extrahepatic biliary microenvironment and generate patient derived cholangiocyte organoids, liver tissue sections from control and PSC patients and serum from control and PSC patients. Bioinformatics was performed on published and in-house human datasets. Experimental techniques include proteomics, multiparameter flow cytometry, ELISA, qPCR, immunofluorescence and western blot.

Results: Olink proteomics revealed PSC patient derived cholangiocyte organoids display a robust secretion of IL-17C. Additionally compared to healthy controls, IL-17C is significantly elevated in the serum of PSC patients. Secondary analysis of published single cell RNA sequencing data on patient derived cholangiocyte organoids demonstrate MR1 (antigen presenting molecule that MAIT cells recognize) upregulation in PSC when compared to non-PSC controls. Western blot and immunofluorescence analysis identified the expression of IL-17RE on MAIT cells. Secondary analysis of published single cell RNA sequencing dataset on PSC patient intrahepatic T cells reveal that IL-17RE gene expression is mainly present in the MAIT cell cluster. Healthy donor derived MAIT cells express a few profibrogenic molecules (by proteomics) including SPARC and TNFSF14 (LIGHT) which are proteins implicated in liver fibrosis by modulating the extracellular matrix and activating stellate cells. MAIT cells release and express SPARC and LIGHT respectively when activated with their cognate antigen MR1-5OPRU.

Discussion/Conclusion: These data suggest an enhanced capacity of cholangiocytes to communicate with and potentially activate MAIT cells in PSC using the T cell receptor (by expressing MR1) and cytokine (IL-17C) mediated pathways; and activated MAIT cells produce profibrogenic mediators. We plan to interrogate and delineate the mechanism of the cholangiocyte-MAIT cell crosstalk in a multi-cellular organoid model which recapitulates the biliary microenvironment. Results of this study has the potential to open new therapeutic strategies for the management of PSC.

3. Secretome from hepatic innate immune cells in activating stellate cells

Ehsan Bahrami (Biberach, DE)

Introduction: Metabolic dysfunction-associated steatohepatitis (MASH) is a heterogenous group of chronic liver diseases characterized by an increasing accumulation of steatosis, liver inflammation, fibrosis and hepatocytes damage. Hepatocyte damage triggers the activation of resident immune cells, such as Kupffer cells (KC), as well as the recruitment of immune cells from the circulation towards areas of inflammation. After infiltration, monocytes differentiate into monocyte-derived macrophages (MoMF) which are functionally distinct from resident KC. Mechanism of secretome crosstalk between various hepatic innate immune cells in activation of Stellate cells is not fully understood

Methods: We herein aim to compare in vitro signatures of polarized macrophages and activated hepatic stellate cells (HSC) with ex vivo derived disease signatures from human NASH subjected to mRNASeq analysis. CD14+ monocytes were isolated from frozen primary human PBMC by MACS and differentiated to MoMF. Immune cell activation was controlled by assessing cytokine secretion patterns by multiplex ELISA and qPCR. By supernatant transfer assay, the fibrogenic potential of activated immune cells on primary human hepatic stellate cells (HSC) was assessed.

Results: IL-14-treated Mac induced TGF-β1 secretion, though supernatant transfer did not induce HSC activation. Interestingly, macrophages stimulated with PMA showed a strong induction of the fibrosis response genes COL10A1 and CTGF, while supernatant of IL-4/IL-13 treated monocytes induced upregulation of COL3A1 in HSC. Supernatant of PMA-activated NK cells had the strongest effect on COL10A1 induction in HSC, while IL-15 stimulated NK cells reduced the expression of COL1A1 and CTGF. Accordingly, pairwise gene-cluster enrichment analysis indicates a continuous loss of IL-4/IL-13 polarized MoMF during NASH disease progression from F0 to F4.

Discussion/Conclusion: Our data indicate that other factors, aside the well-known cytokines and chemokines, might have stronger potential in activation of HSCs, indicating a more complex interaction among hepatic immune cells in liver fibrosis progression.

4. A case with seronegative autoimmune hepatitis: Too often missed or delayed in clinical practice

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Introduction: Autoimmune hepatitis (AIH) is a rare liver disease (0.7 to 2 per 100,000 people) which is characterized by the presence of autoantibodies. However, an average of 10% of AIH cases have autoimmune pathology but lack autoimmune serology. For such seronegative AIH (snAIH) cases, there is currently no established diagnostic algorithm for diagnosis and improper or delayed diagnosis of snAIH and can result in progression to cirrhosis. Here, we present a case with cirrhosis with seronegative autoimmune hepatitis diagnosed by liver histopathology and confirmed by steroid therapy

Methods: A 38-year-old male patient presents with abdominal pain and yellowish colour in urine and sclera for the last 15 days. The left abdominal distension has been a complaint for the last 4 months (because of enlarged spleen). He had type 2 diabetes mellitus (DM). The patient's body mass index (BMI) is 28 kg/m2. An transabdominal ultrasound and a CT scan with contrast showed lobulation of the liver contours, segmental hypertrophy of the left lobe, and atrophy of the right lobe, a portal vein 13 mm and spleen 210 mm with mild ascites in the pelvic area. İn his laboratory tests, the following results were obtained: Hb 12.7 g/dl (lower than normal), PLT: 68.000 (low), and WBCC: 3560 mm3 (low), INR:1.1. Serum levels of ALT: 139 IU/l, AST: 134 IU/l, ALP: 267, GGT: 694 IU/l (all higher than normal). All serology tests were negative for hepatitis B and C, ASMA, AND, LKM-1 and AMA. IgG and IgM were mildly elevated. Gaucher test was also negative.

Results: Liver biopsy performed and showed that: cirrhosis (f4 fibrosis), interface periportal and periseptal hepatitis, lymphoplasmacytic necroinflammatory infiltrate, and plasma cell infiltration. Steroid therapy was started with 30 mg/day prednisolone and ursodeoxycholic acid 1500 mg/day orally. After 30 days, she is on a good condition and normal serum ALT, AST, ALP, and GGT.

Discussion/Conclusion: There is no definitive test currently available for the diagnosis of snAIH. The diagnosis of snAIH may be made on the basis of the combination of a hepatic

pattern of serum aminotransferases, lack of positive autoantibodies, lack of elevation of total IgG, typical histological findings, immunogenetic background. Typical hepatic features of pathology include cirrhosis (19–83%), interface periportal or periseptal hepatitis (75–83%), lymphoplasmacytic necroinflammatory infiltrate, and plasma cell infiltration (17–50%). Advanced histological stage (f3, f4) is more commonly seen in snAIH than classical AIH as happened in our case. Three-month treatment trials with corticosteroids should be considered in all patients regardless of the serological findings to further confirm diagnosis in patients with a positive response. We gained improvement on hi general condition and laboratory results.

5. Hepatocyte oxidative stress and inflammatory responses enhance osteoclast function in a human 3D in vitro liver-bone co-culture system

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Introduction: Reactive oxygen species (ROS) are inevitable byproducts of drug metabolism in the liver. When antioxidant defenses cannot balance the ROS generated, oxidative stress occurs, leading to inflammation and liver damage. Diclofenac administration can cause liver damage and impair bone health. We hypothesize that ROS and metabolic products associated with diclofenac metabolism in the liver could negatively impact bone homeostasis. Our research aims to explore how diclofenac affects bone homeostasis through the liver-bone axis.

Methods: Osteoblast and osteoclast precursor cells on the scaffold were co-cultured indirectly with human hepatic progenitor HepaRG spheroids. 6 µM diclofenac was exposed daily to the system for up to 28 days. In addition, bone scaffolds were directly exposed to 5 pg/ml IL-6 or 600 nM 4-hydroxy-diclofenac (4-OH diclofenac). Interleukin-6 (IL-6), receptor activator of nuclear factor kappa-B ligand (RANKL), and osteoprotegerin (OPG) protein levels in supernatant were detected. ROS, total glutathione (GSH), resazurin conversion, total DNA, alkaline phosphatase (AP), tartrate-resistant acid phosphatase (TRAP), and scaffold mineral content were analyzed.

Results: Our results showed that diclofenac upregulates TRAP ($p = 0.0012$) and downregulates bone scaffold mineral content ($p < 0.001$) in the liver-bone system. 4-OH diclofenac did not negatively affect bone homeostasis. Diclofenac upregulated ROS levels ($p = 0.32$) and reduced total GSH levels in HepaRG spheroids ($p = 0.08$). IL-6 protein levels were upregulated with diclofenac exposure, resulting in RANKL: OPG increase ($p < 0.001$). TRAP increased ($p < 0.001$) and mineral content decreased ($p = 0.09$) with exposure of bone scaffold to IL-6.

Discussion/Conclusion: The increased ROS and decreased GSH induced by diclofenac in liver cells could contribute to chronic inflammation, leading to increased levels of IL-6. The upregulation of inflammatory factors leads to an imbalance of RANKL: OPG, resulting in the activating of osteoclasts. This suggests that diclofenac can affect bone health through hepatic oxidative stress and inflammation.

6. Mass spectrometry-based untargeted metabolomics study of non-obese individuals with non-alcoholic fatty liver disease

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Introduction: Non-alcoholic fatty liver disease (NAFLD) is a disease characterized by the accumulation of excessive fat in the liver, which can lead to fibrosis and has an increasing prevalence. NAFLD requires non-invasive diagnostic biomarkers. While typically observed in overweight individuals, it can also occur in non-obese/non-overweight individuals. Comparative studies on non-obese NAFLD patients are scarce. This study aimed to conduct a using liquid chromatography-high resolution mass spectrometry (LC-MS/MS)-based metabolic profiling of non-obese NAFLD patients and healthy controls.

Methods: The patient group consisted of 27 individuals with NAFLD, while the healthy control group included 39 individuals. Both groups were between 18 and 40 years old, had a BMI of less than 25 and had alcohol consumption less than 20 g/week for men and 10 g/week for women. Serum samples were collected and analyzed using LC-MS/MS. The data were analyzed using the TidyMass and MetaboAnalyst.

Results: The LC-MS/MS analyses detected significant changes in D-amino acid metabolism, vitamin B6 metabolism, apoptosis, mTOR signaling pathway, lysine degradation, and phenylalanine metabolism pathways in non-obese NAFLD patients. Significant changes were also observed in the metabolites D-pantothenic acid, hypoxanthine, citric acid, citramalic acid, L-phenylalanine, glutamine, and histamine-trifluoromethyl-toluidide, β-hydroxymyristic acid, DL-Lactic acid, and 3-methyl-2-oxopentanoic. Overall, the study provides valuable insights into the metabolic changes associated with non-obese NAFLD patients and can contribute to the development of non-invasive diagnostic biomarkers for NAFLD.

Discussion/Conclusion: This study sheds light on the metabolic changes in non-obese NAFLD patients. Further research is needed to better understand the metabolic changes associated with NAFLD and to develop effective treatment options.

7. The role of inhibitor of differentiation proteins in hepatocellular carcinoma

Hanna Ehnis (Erlangen, DE), Claus Hellerbrand (Erlangen, DE)

Introduction: Inhibitor of differentiation (ID) proteins are involved in many cellular processes regulating proliferation and differentiation. In vertebrates four different isoforms (ID1–ID4) are known. Altered ID levels have been found in different types of cancer. Information regarding the expression of the different ID proteins in hepatocellular carcinoma (HCC) is only sparse. The aim of this study was to analyze the expression and function of ID1–4 in HCC.

Methods: Different human HCC cell lines (Hep3B, HepG2, PLC and Huh7) were analyzed by RT-qPCR and Western blotting. The expression of the IDs was suppressed using RNAi technology.

Results: ID1, ID2 and ID3 mRNA and protein expression is significant higher in different human HCC cell lines compared to primary human hepatocytes. Suppression of IDs in HCC cells showed reduced colony formation as well as reduced cell proliferation. In silico analysis of TCGA-LIHC human HCC tissues revealed that enhanced ID1, ID2, ID3 and ID4 expression correlates with poor overall and progression free survival.

Discussion/Conclusion: Our in vitro analyses indicate that the ID proteins exhibit protumorigenic effects on HCC cells. The inverse correlation of IDs with patients' survival further suggests the ID proteins as tumor promoter in HCC. Together, our study indicates the IDs as potential diagnostic markers and therapeutic targets in HCC patients.

8. Bone morphogenetic protein 13 exhibits prometastatic effects on melanoma cells

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Introduction: Several bone morphogenetic proteins (BMPs) have been shown to act as tumor promotors in different types of cancer including melanoma. Recently, our group has identified hepatic stellate cells as the major producer of BMP13 in fibrotic livers and demonstrated a protumorigenic effect of BMP13 on HCC cells. The liver represents an attractive niche for metastasis of numerous tumors including melanoma and although the molecular mechanisms are still largely unknown, HSCs have been shown to be in close contact with tumor cells in the hepatic environment. The aim of this project was to investigate the effects of BMP13 on melanoma cells.

Methods: Different human melanoma cell lines (MV3, MelIm and SkMel28) were stimulated with recombinant BMP13 (rBMP13).

Results: Stimulation of melanoma cells with rBMP13 caused a time- and dose-dependent induction of the expression of the transcription factors inhibitor of differentiation 1, 2 and 3 (ID1, 2 and 3), known targets of BMP signaling and tumor promotors in various cancers. Consistent with this, rBMP13-stimulation induced Smad1/5/8 phosphorylation as well as reduced expression of cyclin-dependent kinases (CDKs) in melanoma cells. In addition, rBMP13 increased proliferation and acted as a chemoattractant in Boyden chamber assays. Inhibition of the BMP type 1 receptors activin receptor-like kinase (ALK) 2, 3 and 6 abolished the effects of rBMP13 on melanoma cells.

Discussion/Conclusion: These data indicate that BMP13 can contribute to the attraction of disseminated tumor cells to the liver and may promote the growth of metastases in the hepatic environment. The protumorigenic effects of BMP13 on melanoma cells appear to be mediated via the receptors ALK 2/3/6. Further studies are required to confirm the potential of BMP13 and ALK 2/3/6 as therapeutic targets against hepatic melanoma metastasis.

9. Autoimmune hepatopathies: What are the epidemiological and evolutionary particularities?

Hela Gdoura (Sfax, TN), Leila Mnif (Sfax, TN), Nabil Tahri (Sfax, TN)

Introduction: Autoimmune hepatopathies are rare diseases primarily affecting women. This is a heterogeneous group of diseases that includes primary biliary cholangitis (PBC), autoimmune hepatitis (AIH), primary sclerosing cholangitis (PSC), as well as all overlapping forms between these different entities. The aim of our work was to specify the epidemiological, clinical, and evolutionary characteristics of autoimmune hepatopathies based on our experience.

Methods: This is a descriptive retrospective study including patients diagnosed with autoimmune hepatopathy collected in our department between January 2018 and December 2023. For each group of patients, we specified the epidemiological, clinical, therapeutic, and evolutionary data.

Results: A total of 58 patients were included, of which 36 cases were PBC (66.7%), 10 cases were PBC-AIH overlap syndrome (18.5%), 8 cases were AIH (14.8%), and 4 cases were PSC (6.89%). The patients were comprised of 52 women (89.65%) and 6 men (10.34%) with a mean age of 57.81 ± 12.5 years (range 25-84). The disease was initiated by edema-ascitic decompensation in 17.85% of cases and by upper gastrointestinal bleeding due to ruptured

esophageal varices in 17.24% of cases. The functional signs were dominated by jaundice in 24 patients (43.8%), pruritus in 22 patients (37.93%), and deterioration of general condition in 15 patients (25.86%). On clinical examination, scratch lesions were present in 29.6% of cases, melasma was observed in 14.8% of cases, xanthomas were found in two patients, splenomegaly was present in 58% of cases, hepatomegaly in 25% of cases, and clinical signs of hepatic insufficiency were present in 24.36% of cases. Endoscopic signs of portal hypertension were present in half of the cases. Ultrasound showed signs of portal hypertension in 64.9% of cases. A good therapeutic response was observed in 72.41% of our patients. Twelve patients experienced edema-ascitic decompensation, and ten patients had gastrointestinal bleeding due to ruptured esophageal varices after a mean follow-up period estimated at 5.6 years. Poor therapeutic adherence was noted in 9 patients. One case was proposed for liver transplantation due to refractory pruritus. The survival rate was approximately 95% at 5 years.

Discussion/Conclusion: In our series, autoimmune hepatopathies were dominated by PBC. Jaundice and pruritus were the most frequent reasons for consultation. The syndrome of portal hypertension was present at the time of diagnosis in half of the cases. Under treatment, the prognosis remains favorable.

10. Liver sinusoidal endothelial cells maintain T cell tolerance induction by in liver fibrosis

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Introduction: Liver sinusoidal endothelial cells (LSECs) play a critical role in mediating T cell tolerance. Previous studies have demonstrated that targeting autoantigen peptides to LSECs using nanoparticles (NPs) can be an effective therapeutic strategy for autoimmune diseases. In liver fibrosis, however, LSECs capillarize and are believed to acquire enhanced immunogenicity. In this study, we explore whether LSECs in fibrotic livers retain their capacity to induce tolerance to ingested autoantigen peptides.

Methods: To assess LSEC tolerance induction in liver fibrosis, the CCl4 and Mdr2KO mouse models of fibrosis were used. LSECs from fibrotic and non-fibrotic livers were isolated, stimulated with LPS or IFN-γ, and the expression of pro-inflammatory cytokines was evaluated. NP targeting to liver cells was assessed by injecting Cy5-labeled NPs, and the ability of LSECs to cross-present antigens was measured using an antibody recognizing the SIINFEKL peptide on MHC-I molecules. Additionally, Treg conversion assays with LSECs from fibrotic livers as antigen-presenting cells were performed. The therapeutic efficacy of autoantigen-coupled NPs in the context of liver fibrosis was tested in two autoimmune disease models: experimental autoimmune encephalomyelitis (EAE) and autoimmune cholangitis.

Results: In liver fibrosis, the production of pro-inflammatory cytokines like IL-6 by LSECs was elevated. Nevertheless, NP were taken up predominantly by LSECs in fibrotic livers, comparable to non-fibrotic controls and LSECs from fibrotic livers exhibited similar antigen cross-presentation abilities. Moreover, the capacity of LSECs to induce Tregs in an antigen-specific manner was similar between fibrotic and healthy livers. Importantly, NP-mediated targeting of autoantigen peptides to LSECs in mice with established liver fibrosis effectively prevented both CD4+ T cell-driven EAE and CD8+ T cell-driven autoimmune cholangitis.

Discussion/Conclusion: Despite the fibrotic changes in the liver, LSECs maintained their ability to induce specific T cell tolerance to autoantigen peptides. This suggests that their tolerance-inducing function is less affected by liver fibrosis than previously believed.

11. Platelet count to mean platelet volume ratio as a survival predictor in patients with hepatocellular carcinoma

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Introduction: Hepatocellular carcinoma (HCC) is a major cause of cancer-related mortality. Identifying simple and easily accessible biomarkers to predict survival is essential for personalized management. Recent studies have suggested that hematological parameters may be prognostic indicators. Our study aims to evaluate the predictive value of the platelet count to mean platelet volume (PLT/MPV) ratio in predicting 18-month survival in patients with HCC.

Methods: This retrospective study included patients diagnosed with HCC on cirrhotic livers between 2000 and 2024 in our department. The platelet count/mean platelet volume ratio was calculated from hematological parameters. Overall survival at 18 months was defined as the outcome at 18 months after diagnosis. Survival analysis using the ROC curve and Cox regression was performed to evaluate the association between the PLT/MPV ratio and survival.

Results: A total of 76 patients were included, mainly male (64.5%) with a mean age of 70.92 years. The most frequent etiology of cirrhosis was viral hepatitis B or C (64.5%). Most patients had a Child-Pugh score of A (53%) followed by CHILD B (35%). Most patients presented with an advanced stage (BCLC C and D) (48.7%). The platelet count to mean platelet volume ratio was significantly predictive of 18-month overall survival with an AUC of 0.69 ($p = 0.016$). A cutoff of 12.9 had a sensitivity of 83%, specificity of 64%, positive predictive value of 43%, and negative predictive value of 92%. Cox regression results showed that a PLT/MPV ratio less than or equal to 12.9 increased the chance of 18-month survival by 2.4 (hazard ratio = 2.4, 95% CI: 1.3–4.2; $p = 0.003$).

Discussion/Conclusion: The PLT/MPV ratio is a simple and easily accessible biomarker to predict 18-month survival in patients with HCC. A low PLT/MPV ratio is associated with a poor prognosis. The integration of the PLT/MPV ratio into HCC management tools could allow for better patient stratification and more personalized treatment adaptation.

12. The HALP score: A new biomarker for prognostic stratification in hepatocellular carcinoma

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Introduction: Hepatocellular carcinoma (HCC) poses a significant public health challenge due to its often poor prognosis. Despite therapeutic advancements, the search for novel predictive biomarkers remains a priority. The HALP score, reflecting nutritional and inflammatory status, has garnered increasing interest in other cancer types. The aim of this study was to evaluate the predictive value of the HALP score for overall survival in patients with HCC.

Methods: This retrospective study included patients diagnosed with HCC on cirrhotic livers between 2000 and 2024 in our department. Overall survival was defined as the time from diagnosis to death. The HALP score was calculated, using: hemoglobin (g/l) * albumin (g/l) *lymphocytes (*109/l) / platelets (*109/l). The predictive value of overall survival at 18 months was analyzed using the ROC curve.

Results: A total of 76 patients were included, primarily male (64.5%) with a mean age of 70.92 years. The most frequent etiology of cirrhosis was viral hepatitis B or C (64.5%). The majority of patients had a Child-Pugh score of A (53%) followed by Child B (35%). Most patients presented with an advanced stage (BCLC C and D) (48.7%). The HALP score was significantly predictive of 18-month overall survival with an AUC of 0.68 ($p = 0.022$). A cut-off of 36.8 had a sensitivity of 78%, specificity of 53%, positive predictive value of 35%, and negative predictive value of 88%.

Discussion/Conclusion: The HALP score could represent a simple and accessible prognostic score for risk stratification in patients with HCC. Larger prospective studies are needed to confirm these findings and evaluate the clinical utility of the HALP score.

13. The ITA.LI.CA score: A new predictor of overall survival in patients with hepatocellular carcinoma

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Introduction: Hepatocellular carcinoma (HCC) continues to be a major public health problem. Despite therapeutic advances, mortality rates remain high.The identification of reliable predictive biomarkers is essential for personalized management. The ITA.LI.CA score, a recently developed prognostic model to assess the risk of death in HCC patients, has shown promising results. The aim of this study was to evaluate the performance of the ITA.LI.CA score in predicting overall survival in a cohort of HCC patients.

Methods: This retrospective study included patients diagnosed with HCC on a cirrhotic liver between 2000 and 2024 at our department. Overall survival was defined as the time from diagnosis to death. The ITA.LI.CA score integrates several factors: tumor size and location, number of tumor nodules, alpha-fetoprotein (AFP) level, Child-Pugh score, and disease stage. The performance of the ITA.LI.CA score was evaluated using receiver operating characteristic (ROC) analysis.

Results: A total of 76 patients were included, primarily male (64.5%) with a mean age of 70.92 years. The most frequent etiology of cirrhosis was viral hepatitis B or C (64.5%). The majority of patients had a Child-Pugh score of A (53%) followed by Child B (35%). Most patients presented with an advanced stage (BCLC C and D) (48.7%). ROC analysis demonstrated that the ITA.LI.CA score was an excellent predictor of 1-year overall survival with an AUC of 0.80 ($p < 0.01$). A score below or equal to an optimal cutoff of 4.5 had a sensitivity of 73%, specificity of 77%, positive predictive value of 63%, and negative predictive value of 84%, and also for 3-year overall survival (AUC, 0.86, $p < 0.01$). An optimal cutoff of 3.5 was defined, offering a sensitivity of 82%, specificity of 80%, positive predictive value of 41%, and negative predictive value of 96%.

Discussion/Conclusion: The ITA.LI.CA score is a powerful prognostic tool for predicting overall survival in HCC patients. Its high performance in terms of sensitivity and specificity suggests that it could be used routinely in clinical practice to stratify patients and tailor therapeutic strategies.

14. Primary biliary cholangitis: Predictive factors for decompensation

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Introduction: Primary biliary cholangitis (PBC) is an autoimmune liver disease for which

the standard treatment is ursodeoxycholic acid (UDCA). This treatment generally helps to slow the progression of the disease and improve survival. However, complications may arise. The aim of our study is to determine the predictive factors for the occurrence of complications during treatment in PBC.

Methods: This is a retrospective study including patients diagnosed with PBC, collected in our department between January 2017 and December 2023. The diagnosis of PBC was based on the biological, immunological, and histological criteria of Paris. UDCA is administered at the recommended dose (13–15 mg/kg/day) to all patients with confirmed PBC. Epidemiological, clinical, therapeutic, and evolutionary data were specified, followed by statistical analysis using SPSS software (p significant if ≤ 0.05).

Results: We collected data from 44 women (95.7%) and 2 men (4.3%) with a mean age of 57.2 ± 12.1 years, ranging from 25 to 84 years. During treatment, 14 patients (30.4%) experienced decompensation during the course of the disease, with 57.2% presenting with edema-ascitic decompensation and 42.8% with hemorrhagic decompensation. Hepatic encephalopathy was noted in 2 patients. The mean time to decompensation from the start of treatment was 36.64 months (1–73 months). At the initial assessment of the disease, the presence of clinical signs of portal hypertension (p = 0.003), esophageal varices (p = 0.005), and hypertensive gastropathy (p = 0.001) were significantly associated with the occurrence of complications during the disease progression. The mean FibroScan value at the time of diagnosis was 22.58 kPa, with extremes ranging from 10.6 kPa to 35.7 kPa. The evaluation by FibroScan after one year of treatment showed a decrease of more than 7.5% from the initial value in 48.7% of cases. During treatment, the persistence of hepatic cytolysis and/or cholestasis was significantly associated with the occurrence of decompensation (p = 0.03; p = 0.004, respectively). Thrombocytopenia, hyperbilirubinemia (≥ 86.7 µmol/l), and hypoalbuminemia (28.5 vs. 36.8), with p values of 0.002, 0.023, and 0.001 respectively, were predictive factors for the occurrence of complications during treatment. A FibroScan value greater than 15 kPa after one year of treatment was a predictive factor for decompensation. For the assessment of one-year survival, the area under the curve was 0.87 for the GLOBE score and 0.90 for the UK-PBC score. For five-year survival, the area under the curve was 0.96 for the GLOBE score and 0.84 for the UK-PBC score.

Discussion/Conclusion: In our series, the occurrence of complications in PBC is more frequent in the presence of clinical-biological signs of portal hypertension and the persistence of hepatic function disturbances during treatment. Monitoring by FibroScan allows for the prediction of complications and therapeutic adjustments

15. The role of stanniocalcin 2 in hepatocellular carcinoma

Paulina Hoefer (Erlangen, DE), Claus Hellerbrand (Erlangen, DE)

Introduction: Stanniocalcin 2 (STC2) is a glycosylated peptide hormone. It is expressed in multiple tissues and plays a critical role in cell proliferation and inflammation. Dysregulation of STC2 expression has been described in different types of cancer, with in part protumorigenic as well as antitumorigenic effects. The aim of this study was to analyze the expression and function of STC2 in hepatocellular carcinoma (HCC).

Methods: STC2 expression was analyzed with quantitative RT-PCR. RNAi technology was applied to suppress STC2 in HCC cells. Kaplan-Meier Plotter, GEPIA, and SurvExpress databases were used for in silico analysis of STC2 in human HCC tissues.

Results: A high STC2 expression was found to correlate with a poor survival rate in HCC through in silico analysis. In a public dataset, higher STC2 expression was observed in tumor samples compared to normal samples. STC2 was significantly higher expressed in

different human HCC cell lines compared with primary human hepatocytes as well as in human HCC tissues compared with corresponding non-tumorous liver tissues. Knockdown of STC2 in HCC cells was established by transfection with siRNA-pools directed against STC2. STC2 depletion in HCC cell lines led to alterations in glycolysis associated genes such as HK1 and LDHa, as well as further genes associated with tumor progression and metastasis such as VEGF and MMP2.

Discussion/Conclusion: Our data indicate a complex role of STC2 in HCC compatible with a protumorigenic role of STC2 in HCC cells. Further studies are required to elucidate the potential of this secreted hormone as diagnostic marker and therapeutic target in HCC.

16. Adipocyte secreted factors induce tumorigenic and prometastatic behaviour of hepatocellular carcinoma cells in vitro

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Introduction: Obesity is characterized by expansion of adipose tissue, resulting in high concentrations of soluble factors from visceral adipose tissue reaching the hepatic sinusoids. Furthermore, obesity is a known risk factor for the development of HCC, but it is only incompletely understood how factors secreted from adipose tissue may influence the progression of HCC.

The aim of the study was to assess whether adipocyte derived factors have protumorigenic effects on HCC cells and to get insights into the effect of enlarged adipose tissue on HCC progression.

Methods: The murine embryonic fibroblast cell line 3T3-L1 was differentiated into adipocyte-like cells to mimic adipogenesis. We generated conditioned medium (CM) of differentiated 3T3 cells. Subsequently, human HCC cells were stimulated with CM to analyze gene expression and functional effects.

Results: Stimulation with CM induced the expression of perilipin-2 (PLIN2), a structural component of lipid droplets, in HCC cells. Furthermore, CyclinD1 and epithelial-mesenchymal transition (EMT) markers such as Snail and Slug were upregulated. Fitting to this, functional analyses showed that adipocyte derived CM induced the proliferation and the migratory activity of HCC cells.

Discussion/Conclusion: Our data indicate that adipocyte-secreted factors promote tumorigenic and prometastatic behaviour of HCC cells. We propose that our in vitro model can be used to further evaluate how obesity and enlarged adipose tissue may promote HCC progression and it may be used to identify protumorigenic soluble factors secreted from adipocytes as potential diagnostic and therapeutic targets for patients with HCC.

17. Immunoregulation and MDSC-mediated T cell paralysis in hepatocellular carcinoma following rVSV-NDV therapy

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Introduction: Hepatocellular carcinoma (HCC) remains a leading cause of cancer-related mortality, characterized by a profoundly immunosuppressive tumor microenvironment that hinders effective immune responses. This study evaluates the efficacy of an oncolytic virus therapy in modulating anti-tumor immunity in HCC. Treatment with a recombinant

chimeric virus, rVSV-NDV, has been shown to mediate therapeutic effects in preclinical models of HCC, although curative responses were not achieved.

Results: Here, we demonstrate robust recruitment of monocytes and effector CD8+ T cells into the liver and tumor in response to rVSV-NDV treatment in an HCC mouse model. Subsequent analyses revealed that infiltrating monocytes differentiate into myeloid-derived suppressor cells (MDSCs), coinciding with T cell infiltration and potentially neutralizing their cytotoxic effects. This transition suggests a mechanism of MDSC-mediated inhibition of T cell function within the tumor microenvironment. In vitro studies demonstrated that HCC cells could drive the differentiation of monocytes into MDSCs, which subsequently suppressed T cell activity via methylglyoxal transfer.

To validate the presence and relevance of these cells in vivo we analyzed scRNA-seq datasets from murine HCC models, not only revealing a population of MDSCs but also indicating a potential intermediate state between mononuclear phagocytes and MDSCs. Current investigations focus on elucidating the induction steps and mechanisms driving the differentiation of mononuclear phagocytes into MDSCs, with implications for understanding their role in shaping the immunosuppressive landscape of HCC and identifying potential therapeutic targets.

Discussion/Conclusion: These findings offer insights into the complex interplay between viral therapy and immune suppression, informing future strategies to overcome resistance in immunotherapy.

18. Expression and function of G protein-coupled receptor 37 in hepatic fibrosis

Seraphine Jochem (Erlangen, DE), Claus Hellerbrand (Erlangen, DE)

Introduction: G protein-coupled receptors (GPRs) play a critical role in various disease processes, including chronic liver disease. GPR37 is an orphan receptor associated with the development and progression of different pathologies, but its role in hepatic fibrosis remains unknown.

The aim of this project was to analyze the expression and function of GPR37 in hepatic fibrosis and hepatic stellate cells, the main cell type responsible for extracellular matrix deposition in chronic liver disease.

Methods: The expression of GPR37 was analyzed at RNA level by RT-qPCR. Furthermore, the expression of GPR37 was suppressed in human HSC cells by RNA interference (RNAi).

Results: GPR37 expression increased during the activation of primary murine and human HSC, which is a key event in the development of liver fibrosis. Accordingly, GPR37 expression was found to elevated in different murine models of hepatic fibrosis. To gain insight into the functional role of GPR37 in hepatic fibrosis, GPR37 was suppressed in HSC using RNAi technology. GPR37 depletion in activated human HSC significantly reduced the expression of alpha smooth muscle actin (α-SMA), a marker of activated HSC, and the fibrosis-associated chemokine C-X-C motif chemokine ligand 1 (CXCL-1).

Discussion/Conclusion: Elevated expression of GPR37 during activation of HSC cells and during hepatic fibrogenesis and first in vitro analysis with GPR37 suppressed HSC indicate this G protein-coupled receptor as a profibrogenic factor. Further studies are required to identify the ligand(s) that act via GPR37 on HSC and to assess the potential of GPR37 as a fibrosis marker and antifibrogenic therapeutic target in patients with chronic liver disease.

19. Neutrophil-to-lymphocyte ratio (NLR) and systemic inflammation index (SII): Predictive inflammatory markers of advanced hepatocellular carcinoma

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Introduction: Hepatocellular carcinoma (HCC) constitutes a significant public health challenge. Early detection of advanced disease is crucial for optimizing therapeutic management. Recent studies have highlighted the potential role of inflammatory markers in cancer progression. Our study aimed to evaluate the prognostic value of the neutrophil-to-lymphocyte ratio (NLR) and systemic inflammation index (SII) in predicting advanced-stage HCC.

Methods: This retrospective study included patients diagnosed with HCC on cirrhotic livers between 2000 and 2024 in our department. The NLR was calculated as neutrophils/lymphocytes, and the SII was calculated as neutrophils × platelets/lymphocytes. A ROC curve analysis was performed to differentiate advanced (BCLC C or D) and less advanced HCC patients using NLR and SII.

Results: A total of 76 patients, predominantly male (64.5%) with a mean age of 70.92 years, were included in the study. The most frequent etiology of cirrhosis was viral hepatitis B or C (64.5%). The majority of patients had a Child-Pugh score of A (53%) followed by Child B (35%). Most patients presented with an advanced stage (BCLC C and D) (48.7%). Results showed that both NLR (AUC, 0.68, p = 0.008) and SII (AUC, 0.69, p = 0.007) were significantly associated with advanced HCC stage. Optimal cut-off values of 3.8 for NLR and 340.5 for SII were identified, allowing differentiation of patients with sensitivities of 48% and 76% and specificities of 85% and 64%, respectively. These results suggest that elevated NLR and SII values are associated with advanced HCC stage.

Discussion/Conclusion: This study suggests that NLR and SII could be promising novel biomarkers for risk stratification in patients with HCC. Integrating NLR and SII into HCC management could identify high-risk patients earlier, enabling tailored therapies.

20. The Metroticket 2.0 score: A powerful predictor of progression-free survival in patients with hepatocellular carcinoma

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Introduction: Hepatocellular carcinoma (HCC) remains a significant challenge in oncology. Identifying robust predictive biomarkers is essential to optimize therapeutic management. The Metroticket 2.0 score, a multifactorial prognostic model, has shown promise in predicting post-transplant mortality in HCC patients. This study aimed to evaluate the prognostic value of the Metroticket 2.0 score in predicting progression-free survival in HCC patients independent of transplantation.

Methods: This retrospective study included patients diagnosed with HCC on a cirrhotic liver between 2000 and 2024 at our department. Clinical, biological, and tumor characteristics were collected. Progression-free survival was defined as the time from diagnosis to disease progression or death. The Metroticket 2.0 criteria were defined as follows: AFP level must be < 200 ng/ml and the sum of the number and size of tumors (in centimeters)

must not exceed 7; if the AFP level is between 200 and 400 ng/ml, the sum of the number and size of tumors must be ≤ 5; if the AFP level is between 400 and 1000 ng/ml, the sum of the number and size of tumors must be ≤ 4. A Cox regression analysis was performed to assess the association between the Metroticket 2.0 score and progression-free survival.

Results: A total of 76 patients were included, primarily male (64.5%) with a mean age of 70.92 years. The most frequent etiology of cirrhosis was viral hepatitis B or C (64.5%). The majority of patients had a Child-Pugh score of A (53%) followed by Child B (35%). Most patients presented with an advanced stage (BCLC C and D) (48.7%). Regarding treatment: only 5 patients underwent surgical resection, 6 patients had percutaneous radiofrequency ablation with a good response, 20 patients (26.3%) had transarterial chemoembolization, 7 patients were treated with Sorafenib. Progression-free survival was significantly higher in the group of patients meeting the Metroticket 2.0 criteria (11 months vs. 7.4 months, p = 0.024), with a 2-fold increased chance of progression-free survival (hazard ratio = 1.9; 95% CI: 1.1–3.3; p = 0.026).

Discussion/Conclusion: The Metroticket 2.0 criteria represent a powerful predictor of progression-free survival in patients with HCC. Integrating the Metroticket 2.0 score into clinical practice could allow for better patient stratification and personalized treatments.

21. Dietary modification with oral probiotics influences regulation of immune cells in MASLD

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Introduction: MASLD is a multi-factorial and insufficiently understood disease, a part of metabolic syndrome with sustainably rising incidence, being the most common chronic liver disease worldwide, affecting approximately 25% of the general population. Immune cells participate in the occurrence and development of the metabolic disorder-related liver disease, and in the MASH stage, immune cell-mediated inflammation could be a key factor in the progress of this disease. However, the influence of diets and dietary interventions including probiotics is still unclear. Multiple studies indicate that fast-food or methionine-choline-deficient diets significantly aggravate risk of MASLD development. Moreover, the concept of gul-liver axis signifies rationale for this study. We hypothesized that dietary interventions with probiotic compositions may have impact not only on intestinal microbiota but also significant influence on immunity and inflammation in MASLD via gut-liver Axis.

Methods: Sixty patients with verified MASLD participated in the study (42 men and 18 women, mean age 57.9 ± 4.8 yrs). Diagnosis and management according to EASL/EASD/EASO Clinical Practice Guidelines EASL-EASD-EASO Clinical Practice Guidelines. Patients were randomized into the following groups: 1st group included all patients before treatment, 2nd group – 23 patients after standard unmodified treatment (EASL/EASD/EASO, 2016, 2023), 3rd group – 17 patients after additional treatment with oral probiotics (Bifidobacterium longum and Enterococcus faecium), 4th group – 20 patients after additional treatment with oral probiotics (Bacillus subtilis and Bacillus licheniformis). Control group included 17 practically healthy subjects. Pro- and anti-inflammatory cytokines: transforming growth factor beta (TGF-β), tumor necrosis factor alpha (TNF-α) and IL-1β were typified (ELISA) before and after 60 days of treatment in all groups.

Results: Both IL-1β and TNF-α levels before treatment were almost twice higher than in practically healthy subjects; TGF-β level was 2.8 times higher than in control. The data ob-

tained was as follows: IL-1β – 92.1 ± 2.8, 70.9 ± 3.5, 65.6 ± 4.2, 72.2 ± 3.7, and 50.7 ± 3.6 for 1st, 2nd, 3rd, 4th group, and control, respectively; TNF-α – 86.1 ± 2.2, 71.7 ± 3.5, 83.3 ± 3.5, 93.7 ± 4.2, and 46.3 ± 4.3, respectively; TGF-β – 150.4 ± 5.0, 142.1 ± 7.4, 72.0 ± 3.9, 63.5 ± 4.1, and 53.1 ± 3.6, respectively. In 2nd group IL-1β and TNF-α levels decreased 22.9% and 16.8% accordingly, with still higher values compared to control group's results. TGF-β level did not statistically differ in the 2nd group after standard treatment. Probiotic administration in 3rd group caused IL-1β level decrease by 28.7%, TGF-β level decrease 2.1-fold with regard to 1st group, but all data were still higher than in control group – 29.4%, 35.6%, accordingly, especially TNF-α level – 79,9%. Probiotic administration in fourth group caused IL-1β level decreasing by 21.6%, TGF-β level decreased 2.4 times and did not differ reliably from the control group; TNF-α level became certainly higher, comparatively to second and control groups.

Discussion/Conclusion: Standard MASLD-related therapy of patients caused significant IL-1β and TNF-α concentrations decrease without clear influence on TGF-β level. Various oral probiotic administration led to statistically valid IL-1 and TGF-β levels decrease, but did not significantly change TNF-α concentration. The idea of selecting the pre- and probiotic compositions study in MASLD for influence liver's immune regulatory function looks promising.

22. Serum sterols profiles reflect altered cholesterol homeostasis in cirrhotic patients with PBC and correlate with response to ursodeoxycholic acid

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Introduction: Primary biliary cholangitis (PBC) is a chronic autoimmune cholestatic liver disease of unknown aetiology. Ursodeoxycholic acid (UDCA) is the first-line therapy in PBC however, some patients present insufficient biochemical response to UDCA, indicating worse disease course and survival. Cholesterol is involved in diverse biological activities and in humans derives from de novo synthesis or intestinal absorption. Here, we aimed to investigate and correlate serum sterols profile and PBC phenotypes.

Methods: Overall, we included 129 patients with PBC (age 32–93 years, 119 women). A total of 44 individuals presented with cirrhosis at diagnosis, 13 were transplanted due to PBC before inclusion. Data on biochemical response to UDCA assessed accordingly to Barcelona criteria were available in 62 (48%) patients. Serum sterol levels were measured by gas chromatography/mass spectrometry (GC/MS) at inclusion. Machine learning (ML) algorithms were used to determine a model that can predict UDCA response.

Results: Cirrhotic patients with PBC demonstrated significantly higher sitosterol ($p < 0.01$), campesterol ($p < 0.01$) and cholestanol ($p < 0.01$) levels as compared to the non-cirrhotic individuals. Patients with cirrhosis showed increased phytosterols:cholesterol ratios but decreased lathosterol:cholesterol ratio (all $p < 0.01$). Desmosterol:cholesterol ratios did not differ between patients with and without cirrhosis ($p > 0.05$). Individuals after transplantation displayed increased serum concentration of 7α-hydroxycholesterol as compared with non-transplanted patients which might reflect increased radical oxidation. The ML Support Vector Classifier model for UDCA response prediction based on 24-hydroxycholesterol, 27-hydroxycholesterol, campesterol, cholesterol, desmosterol, lanosterol, stigmasterol, revealed high accuracy, precision and sensitivity (all > 80%) in predicting non-response to the therapy.

Discussion/Conclusion: PBC patients with liver cirrhosis are characterized by significantly decreased cholesterol synthesis and increased sterol absorption as compared to non-cirrhotic individuals. Biochemical response to UDCA therapy correlates with serum levels of selected sterols.

23. Lysine-specific demethylase 1 (LSD1) modulates hepatic lipid metabolism in metabolic dysfunction-associated fatty liver disease

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Introduction: Metabolic dysfunction-associated fatty liver disease (MAFLD) is a prevalent condition affecting approximately 30% of adults worldwide. It is characterized by hepatic steatosis, obesity, type 2 diabetes, and hypertension. Emerging evidence suggests that epigenetic alterations play a pivotal role in the development of MAFLD. Lysine-specific demethylase 1 (LSD1) stands out as a key player in regulating chromatin structure and transcriptional control through the demethylation of histone 3 lysine 4 or lysine 9. Transcriptomic and proteomic analyses have revealed alterations in lipid metabolism gene expression profiles, indicating the involvement of LSD1 in MAFLD pathogenesis.

Methods: To investigate the role of LSD1 in MAFLD development, we employed a high-fat diet mouse model. Systemic and hepatocyte-specific LSD1 inhibition were achieved using a LSD1 inhibitor or liver-specific conditional transgenic LSD1 mutation, respectively, after administration of adeno-associated virus (AAV). RNA-seq from these livers and adipose tissue showed a dysregulation in the chemokine expression profile. Additionally, primary hepatocytes were isolated to further explore the functional significance of LSD1 in MAFLD.

Results: Liver histology demonstrated a significant reduction in steatosis, accompanied by notable weight loss and decreased liver enzyme values after systemic LSD1 inhibition. Notably, hepatocyte-specific LSD1 inactivation also resulted in diminished fat accumulation, although to a lesser extent compared to systemic pharmacological LSD1 inhibition. Transcriptomic analysis of liver tissue from systemic LSD1-inhibited mice revealed marked alterations in lipid metabolism, particularly in genes involved in lipid transportation. Furthermore, chemokine expression profiles were reduced after LSD1 inhibition that favors the decrease in steatosis. Moreover, single nuclei-RNA sequencing from liver-specific LSD1 inactivation revealed an imbalance in proteostasis and stress response.

Discussion/Conclusion: This study underscores the crucial role of LSD1 in MAFLD development. By altering hepatic lipid metabolism, proteostasis and chemokine expression profile

24. Predictive factors for degeneration in compensated post-viral cirrhosis

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Introduction: Hepatocellular carcinoma (HCC) occurs on cirrhosis in 85–95% of cases, and the risk of degeneration is higher in cases of post-viral etiology. The aim of our work was to identify risk factors for the degeneration of initially compensated post-viral cirrhosis.

Methods: Retrospective, descriptive study of all patients followed for post-viral B and/or C compensated cirrhosis between 2010 and 2018.The incidence of HCC occurrence and factors predictive of hepatic degeneration were determined.

Results: Sixty patients were included in our study, including 33 men with a sex ratio of 1.22. The mean age of the patients was 62.05 ± 12.60 years. The etiology of cirrhosis was post-viral C in 70% of cases; cirrhosis was classified as Child A in 88.1%. 33 patients (55%) received antiviral treatment. Sustained virological response was noted in 81.81% of treated patients. During follow-up, 41.66% of patients developed hepatocellular carcinoma after a mean follow-up time of 40.56 ± 43.49 months. Of these patients, 9 had chronic hepatitis B (36%). The cumulative probability of developing hepatocellular carcinoma was 68%, 80% and 92% at 3, 5 and 10 years respectively. Predictive factors for degeneration were advanced age (p = 0.05), cholestasis (p = 0.02), failure to start antiviral therapy (p = 0.01) and lack of virological response (p = 0.02).

Discussion/Conclusion: In our study, the risk of HCC on post-viral cirrhosis was high and associated with non-eradication of the virus.

25. Important roles of HBe and HBs antigens in the evasion of endogenous innate immune responses in hepatitis B virus infection

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Introduction: Hepatitis B virus is a DNA virus with no apparent cytotoxicity but can cause persistent infection. Despite the development of an effective vaccine, an estimated 2 billion people worldwide have evidence of past or present HBV infection; 300 million people are chronic HBV surface antigen carriers and are at high risk of developing hepatocellular carcinoma and liver cirrhosis. 20–30% of the chronically infected patients will develop complications associated with HBV infection, causing more than 700,000 deaths annually. It is proposed that HBV controls or evades endogenous immune responses, whereas activation by exogenous stimuli results in an effective antiviral signalling. Immune evasion occurs not only through pattern recognition receptor signalling, but also through control of cytokine and interferon responses.

Methods: PMH cells were transfected with plasmids of interest pHBe or pHBs for 48h. PRR were stimulated at various time points using their respective ligands and gene expression, luciferase activity and nuclear translocation were measured.

Results: HBeAg and HBsAg suppresses gene expression of antiviral proinflammatory cytokines upon TLR2 stimulation.The antigens also suppressed gene expression of antiviral IFNs upon TLR3 and RIGI/MDA5 stimulation. We also observed that HBeAg and HBsAg significantly suppressed NFkB activation upon TLR2, TLR3 and RIGI/MDA5 stimulation, whilst the activity of ISRE was significantly suppressed upon TLR3 stimulation. NFkB nuclear translocation was observed upon stimulation of TLR2, TLR3 and RIGI/MDA5.

Discussion/Conclusion: HBeAg and HBsAg antigens repress the expression of pro-inflammatory cytokines and anti-viral interferons. The antigens also reduce the activation of ISRE, they suppressed the translocation and activation of transcription factor NFkB.

26. Bile acid receptor Tgr5 as a modulator of macrophage immunometabolism during infection

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Introduction: Tgr5 (Gpbar-1), a membrane-bound bile acid receptor, regulates metabolism by enhancing energy expenditure and glucose control. Primarily expressed in monocytes and macrophages, Tgr5 activation has immunomodulatory effects. This study explored

Tgr5's role in macrophage metabolic modulation during Listeria monocytogenes (L.m.) infections.

Methods: The RNA-seq approach was used to investigate the differential expression of transcripts involved in metabolic pathways in L.m.-infected bone marrow-derived macrophages (BMDMs). Glucose uptake was determined using 2-NBDG and measured by FACS. Glycolytic and oxidative phosphorylation capacity was measured by a Seahorse analyzer. BMDMs were treated with 5mM 2-hydroxycitrate (2-HC) followed by 2 or 6 h with L.m. Expression of cytokines in treated BMDMs were quantified by qPCR.

Results: The RNA-seq analysis indicated that gene expression of pathways, including glycolysis and oxidative phosphorylation, was distinct between WT and Tgr5-/- BMDMs after infection. Tgr5 deficiency caused reduced glycolytic rates and glucose transporter type 1 (Glut1) expression in macrophages after stimulation with L.m. Using RNA-seq, the ATP-citrate lyase (ACLY) expression was reduced in L.m.-stimulated Tgr5-deficient BMDMs. The inhibition of acetyl-CoA generation reversed the suppression of Tgr5-dependent pro-inflammatory cytokine.

Discussion/Conclusion: The differential expression of genes involved in glucose metabolism and oxidative phosphorylation in WT and Tgr5-/- macrophages, along with the altered metabolic profile of Tgr5-/- macrophages following infection, underscores the supportive function of Tgr5 in enhancing immune response.

27. Identification and validation of gene expression pattern in a cell culture model of Wilson's disease

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Introduction: Wilson disease (WD) is an autosomal recessive disorder caused by a defect in the ATP7B gene. ATP7B codes an intracellular copper transporter that is highly expressed in the liver. Dysfunction of ATP7B leads to cellular copper accumulation, culminating in transcriptional changes of ion transporters, disruption of mitochondrial activity, autophagy and finally cell death.

The aim is to reanalyse the RNA sequencing data (GSE107323) to compare the transcriptional response to copper stimulation in wild type and ATP7B knockout hepatoma cells (HepG2) under the same cultivation conditions and to find novel gene groups related to ion transport, autophagy and inflammation that are differentially regulated.

Methods: Multiple component analysis of GEO data (GSE107323) was performed. Treatment of HepG2 WT and HepG2 ATP7B-KO cells with CuCl2 was examined to measure cell viability (CCK8-Assay) and transcriptional changes of various genes related to cell intrinsic immunological signals and autophagy.

Results: Multiple component analysis identified gene expression pattern in the ATP7B knockout cells that are associated with endoplasmic reticulum (ER) stress, autophagy, ion transport, immunological signals and liver functions. Quantitative PCR partially validated the expression of selected genes.

Discussion/Conclusion: ATP7B knockout cells responds with increased autophagy to prevent copper-associated apoptosis. The present expression analysis indicated that next to metal ion transport and autophagy also genes involved in inflammation and liver function were affected.

28. Schistosoma mansoni egg-derived antigens stimulate metabolic activity in the liver and colon by engaging insulin-like growth factor-1 receptor signaling pathways

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Introduction: Schistosomiasis is a parasitic disease that affects more than 250 million individuals worldwide. Compelling evidence suggests that schistosome eggs, rather than adult worms, are responsible for significant tissue damage. S. mansoni eggs have been shown to induce metabolic exhaustion and redox imbalance, which are critical for the integrity of hepatocellular DNA. However, the precise mechanism by which egg antigens transmit signals into host cells remains unclear. In this study, we investigated whether S. mansoni egg antigens regulate the metabolic activity of hepatocytes and enterocytes through the insulin/IGF-1 receptor pathway.

Methods: Eight-week-old mice were infected with S. mansoni cercariae using the paddling technique. We employed RT-PCR array, western blotting, and immunohistochemistry to analyze markers of insulin/IGF-1 receptor signaling in liver and colon. Additionally, we conducted functional experiments using colon epithelial cell lines, which involved western blotting and assessment of AP-1 promoter activity.

Results: The results showed that S. mansoni infection led to a significant upregulation of the expression of genes such as Igf2, Dok, Aebp1, Leptin, and Akt3, with Serpine1 being the most strongly induced. Conversely, infection caused a decrease in the expression of Sos1, Irs1, and Gck, with G6pc being the most strongly downregulated. Additionally, the activation of the insulin/IGF-1 receptor was pronounced in the perigranulomatous hepatocytes. Mechanistic experiments utilizing the insulin/IGF-1 receptor inhibitor BMS 536924 revealed that S. mansoni egg antigens induced the activation of the insulin/IGF-1 receptor signaling cascade, including the activation of the proto-oncogene c-Jun.

Discussion/Conclusion: In conclusion, our findings demonstrate that S. mansoni soluble egg antigens modulate the insulin/IGF-1 receptor signaling pathway in both murine and human hepatocytes and enterocytes, leading to an inhibition of gluconeogenesis. Thus, the parasite's soluble egg antigens may enhance insulin sensitivity in the host. However, the concurrent activation of the proto-oncogene c-Jun through this signaling pathway raises the possibility of potential morbidity associated with this mechanism.

29. Is gluten-free diet a guarantee for healthy diet and optimal weight?

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Introduction: Coeliac disease (CD) is an immune-based enteropathy characterised by a wide range of clinical symptoms, a specific serum antibody response and varying degrees of damage to the mucosa of the small intestine due to gluten intake.

The main therapy for treating CD as well as preventing complications is a lifelong, strict gluten-free diet (GFD). General population often uses GFD as a tool for weight loss, even though it is not scientifically proven to have that effect. On the contrary, there are a number of concerns about the health consequences of following a GFD, particularly the possibility that a GFD could promote overweight or obesity. A large amount of data has been published, with some studies showing an effect of a GFD in underweight patients who

experience a recovery of weight to reach their ideal weight, as well as propensity toward weight gain and obesity, which has been showed in other studies. Furthermore, the nutritional quality of a gluten-free diet continues to affect vitamin and mineral status of individuals with coeliac disease.

Ultraprocessed foods (UPF) are products of food technology, formulated from 5 or more industrial ingredients and containing little or no whole food. Due to their high caloric content, low nutritional quality as well as high glycemic load, they are considered harmful to human health. Furthermore, they often contain additives. Food industry produces various gluten-free options whose consumption has increased throughout the world. Diverse studies correlated the consumption of UPF with the increase in chronic non-communicable diseases in children and adults. Maintaining a strict GFD may affect the quality of life of children with celiac disease (CD) and promote a less healthy diet by substituting gluten-containing foods with ultra-processed gluten-free foods, which may lead to overweight and obesity. So, the aim of this research was to assess intake of ultraprocessed food in this specific patient population.

Methods: This pilot project was conducted with 38 paediatric celiac patients who were examined in June 2024 as part of the medical and nutritional consultation at the Department of Paediatrics at the University Hospital Split. The average age was 10.8 years and the subjects were 25 (65.79%) girls and 13 (34.21%) boys. To assess dietary habits, a validated Food Frequency Questionnaire for estimating dietary contributions of NOVA groups in the subjects' diet with a special emphasis on ultra-processed foods. Data on age, body weight and height were obtained from the patients' medical records. In addition, the body mass index (BMI) and the Z-score of the body mass index value were calculated using the WHO AnthroPlus application tool.

Results: Based on the z-score (BMI), 21.5% of patients were underweight, 60.53% were of normal weight and 18.42% were overweight. In the NOVA groups, several products were consumed twice or more per week: packaged cookies (31.6%), ice cream (42.1%) and chocolate (31.6%). 31.6% of the included patients eat spreads (such as hazelnut spread or peanut butter), 34.2% consume packaged sweet and savoury snacks and 28.9% of our patients consume soft drinks (e.g. iced tea, cola) more than once a week. Interestingly, 28.9% of children consume sugar daily and 13.2% of children consume honey or maple syrup. On the other hand, 68.4% of our patients were found to never consume baked pastries (such as croissants, donuts and Danish pastries) and chocolate-based drinks.

Discussion/Conclusion: An unbalanced gluten-free diet with high consumption of ultraprocessed food and an unhealthy pattern with less physical activity results in a worse inflammatory profile which can lead to multiple comorbidities including type II diabetes mellitus, cardiovascular disease and several forms of cancer. In the present pilot study it was noticed that only consumption of ultraprocessed food which has gluten-free options in Croatian shops (e.g. chocolate) were often consumed. On the other hand, ultraprocessed food with no gluten-free options in Croatia (such as baked pastry) was rarely consumed.

Considering the influence of the media and society at this early stage of development, these results point to the need for structured nutritional education for newly diagnosed coeliac patients, but also for patients who were diagnosed some time ago and their parents. Particular attention should also be paid to the inclusion of UPF and its healthier options.

30. Hobit+ liver resident lymphocytes in MASH-HCC

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Introduction: Resident-like CXCR6+ CD8 T cells accumulate in metabolic dysfunction-associated steatohepatitis (MASH), induce auto-aggression and might be the cause of limited anti-tumor surveillance in immunotherapy treated MASH induced Hepatocellular carcinoma (HCC). However, the generation and maintenance of this population and the different role of other liver resident adoptive and innate lymphocytes remain delusive in the context of MASH-HCC. Homolog of Blimp1 in T cells" (Hobit) expression represent the resident population of lymphocytes including tissue resident memory T cells (TRM), natural killer T cells and resident NK cells in the liver. By using Hobit restricted reporter (Zfp683-tdTomato-cre) and deleter (Rosa26-DTA) systems, we are investigating the different role of resident lymphocytes in MASH and HCC. In our choline deficient high fat diet (CDHFD) MASH model, we found that type 1 innate lymphoid cells (ILC1) are the major accumulating subset of Hobit+ resident lymphocytes by flow cytometry. On the other hand, Hobit+ CD8 TRM represents a distinct inactivated population comparing to the highly expanded activated CXCR6+Hobit- CD8 T cells. More importantly, depletion of Hobit+ cells ameliorates MASH progression and HCC development with reduced auto-aggressive T cells in the liver. We are further investigating the direct or indirect function of ILC1 and the TRM differentiation trajectory using fate mapping transgenic mice and single cell RNA and TCR sequencing.

31. Liver macrophages release IL-6 and TNF-alpha upon ADAM17 and EGF-receptor activation by augmenter of liver regeneration (ALR)

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Introduction: The liver, in contrast to other organs, holds an unique capacity to regenerate after injury. While resting hepatocytes reside in G0 phase, they have to be primed by e.g. IL-6 and/or TNFα entering the G1 phase of the cell cycle. ALR (augmenter of liver regeneration), was discovered to be expressed early and at high levels in vivo under circumstances of liver regeneration and in liver diseases such as liver failure. Additionally in vivo and vitro studies showed an exclusive but mitigated direct effect of ALR on hepatocyte proliferation of regenerating livers.

Methods: Therefore, we hypothesize, that ALR affects hepatic cells other than hepatocytes such as liver macrophages. We investigated the molecular signalling pathways in macrophages upon ALR treatment, since our lab discovered quite recently that ALR stimulates hepatocytes via G-protein coupled receptor (GPCR) and ADAM17/TACE induction subsequently followed by EGF-R activation.

Results: Treatment of RAW 264.7 cells (mouse macrophage cell line) with ALR (100 ng/ml, 6 h) resulted in significant increased mRNA expression of IL-6 and TNFα. Furthermore, performing ELISA assays we found enhanced release of IL-6 and TNFα into supernatants of mouse Kupffer-cells and human THP1 macrophages treated with ALR (100 ng/ml) for 24 h or 6 h, respectively (LPS or IL-1β as positive control). By using specific inhibitors we could demonstrate at least that ALR signalling is mediated by EGF-R and ADAM17 activation,

since IL-6 release of Kupffer-cells is reduced after ALR treatment in presence of AG1478 or GW280264X, respectively. Furthermore, p38 MAPK inhibition by SB203580 suppressed mRNA expression of IL-1β in RAW cells.

Discussion/Conclusion: In conclusion, ALR induces IL-6 and TNFα, both inductors of hepatocyte priming, by activating EGF-R, ADAM17 and p38. EGF-R might be trans-activated by membrane bound ligands, which are liberated by ADAM17. ADAM17 is activated via phospho-src by GPCR induction through ALR, as shown before in epithelial cells.

32. Results of combined prophylaxis in preventing hepatitis B virus recurrence in liver transplant recipients – Insights of a single-center experience

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Introduction: Hepatitis B virus (HBV) recurrence poses a significant concern post-liver transplantation, as it can lead to graft failure and even mortality. While prophylaxis plays a pivotal role in preventing HBV recurrence, optimal strategy is still debatable. We aimed to assess the efficacy and safety of the combined prophylactic strategy in preventing HBV recurrence after liver transplantation.

Methods: We conducted a retrospective study on twenty-two liver transplant recipients, followed in our unit between 2016 and 2024. All patients had a documented history of hepatitis B virus (HBV) infection prior to transplantation and received a combined prophylaxis regimen consisting of anti-HB immunoglobulins (HBIg) and nucleoside/nucleotide analogues after transplantation. Clinical data, pre-transplant HBV viral load, immunosuppressive regimen, and post-transplantation follow-up data were collected. Virological outcomes were evaluated through regular assessments of anti-HBs antibody titers, HBs Ag and HBV-DNA levels. Additionally, graft function assessments were performed at routine intervals post-transplantation.

Results: Among the twenty-five liver transplant recipients in this study, 18 had undetectable HBV-DNA levels at transplantation. All 25 patients received combined prophylaxis consisting in intravenous HBIg during the anhepatic phase and initial months after transplantation, followed by subcutaneous HBIg, combined with oral nucleoside/nucleotide analogues. No HBV recurrence was observed during follow-up, which ranged from two to ninety-two months. Serological and virological assessments remained negative for HBV, with protective anti-HBs antibodies levels. Liver function tests showed preserved graft function in all cases and no HBV-related hepatic complications. No adverse events were recorded.

Discussion/Conclusion: Our findings underscore the excellent efficacy and safety of the combined prophylactic regimen in preventing HBV recurrence following liver transplantation. However, it is essential to acknowledge the drawbacks of our current strategy, including the financial burden and the need for long-term patient adherence. Overcoming these challenges may involve exploring personalized treatment approaches tailored to individual patient characteristics and risk factors.

33. Factors secreted by adipocytes induce the tumorigenic and prometastatic properties of melanoma cells

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Introduction: Melanomas frequently metastasise to the liver and obesity is described to be a potential risk factor for melanoma metastasis and poor prognosis of patients with surgical resection of hepatic metastases. Characteristic for obesity is increased adipose tissue that secretes soluble factors, which get to the liver via the portal vein.

The aim of the study was to get further insights whether adipocyte derived factors promote tumorigenicity of melanoma cells and hepatic metastasis.

Methods: Adipogenesis can be mimicked in vitro by using the 3T3 model, consisting of the murine embryonic cell line 3T3-L1, that can be differentiated to adipocytes and pre-adipocytes. After differentiation we collected conditioned medium of adipocytes. Adipocyte maintenance medium was used as control. Afterwards, different human melanoma cell lines were stimulated with CM or control medium to analyse gene expression, functional effects and activation of protumorigenic signalling pathways.

Results: Treatment with CM led to induced expression of vascular endothelial growth factor (VEGF), which is a known pro-angiogenetic factor and a known marker for poor prognosis of melanoma patients with hepatic metastases. CM stimulation also increased the expression of EMT markers like SLUG. Functional analyses showed that adipocyte CM induced proliferation of melanoma cells. Additionally, CM led to higher migratory activity of melanoma cells in boyden chamber assays and activation of protumorigenic signaling pathways.

Discussion/Conclusion: Secreted factors from adipocytes promote protumorigenic and prometastatic characteristics of melanoma cells, which provides a potential mechanism how obesity and enlarged visceral adipose tissue promote (hepatic) metastasis.

The in vitro system may be used to further characterise adipocyte derived factors as potential diagnostic and therapeutic targets for patients with (hepatic) metastasis.

34. The complex interplay of cytokines, immune cells, and microbial endotoxin in experimental model of hepatic injury signifies liver's role in immune regulation

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Introduction: Liver is an important immune organ, responsible for the production of acute phase proteins, complement components, various cytokines and chemokines, and contains diverse populations of resident immunocompetent cells. Previous studies suggest that liver failure compromise the immune surveillance function of the liver through damage of the reticulo-endothelial system and synthesis of innate immunity proteins. Moreover, liver injury causes extensive systemic immunological pro-inflammatory response characterized by significant growth of IL-1β, TNFα, IL-6 and other pro-inflammatory mediators. Worth noting that microbial endotoxins cause quite similar immune reactions, therefore we hypothesize that this effects in liver injury may combine and/or even multiply. The aim of the study is to determine the possible vicious circle of immunologic changes following liver injury.

Methods: Liver injury was experimentally modelled intraoperatively in 27 adult Wistar rats under general anaesthesia; sutures were applied immediately. In addition, 35 similar rats received S. typhimurium standard endotoxin intraperitoneally. Liquid chromatography and ELISA were used for study of cytokines levels in liver tissue homogenates taken 24 hrs after injury. Bioethics requirements were strictly obeyed.

Results: Isolated aseptic liver injury alone caused minor changes in cytokines levels in liver homogenates compared to normal values obtained in previous experiments: IL-1β grew insignificantly (55.72 ± 8.30 pg/g of homogenated hepatic tissue under liver injury compared to 45.37 ± 5.82 pg/g in healthy control rats, $p > 0.05$); TNFα – 39.26 ± 4.89 pg/g and 47.63 ± 6.47 pg/g, $p > 0.05$, respectively; gamma-interferon (γ-INF) – 112.9 ± 7.62 pg/g and 123.9 ± 10.75 pg/g, $p > 0.05$; TGF-β1 – 204.5 ± 12.17 pg/g and 196.2 ± 6.42 pg/g, $p > 0.06$. Intraperitoneally added LPS-endotoxin caused dramatic increase of cytokines in liver tissue: IL-1β increased over 80% to 74.27 ± 8.09 pg/g, $p < 0.01$; TNF-α grew 45% to 56.91 ± 6.53 pg/g, $p < 0.05$; γ-INF level (419.16 ± 30.68 pg/g) raised 3.7 times compared to liver injury without addition of endotoxin, $p < 0.001$. Surprisingly, under additional exposure to endotoxin TGF-β1 level remained almost unchanged, with unreliable growth tendency – 236.16 ± 25.68 pg/g, $p > 0.05$.

Discussion/Conclusion: It is generally accepted that any type of liver injury due to disease or trauma is generally accompanied by proinflammatory tendencies. However, proinflammatory stimulation in lever injury is generally insignificant both in terms of clinical and bench points of view in case of isolated aseptic and immediately cured trauma. However, this presents ideal, theoretic condition; raised endotoxin level is inevitable in case of real clinical situation in relation to trauma. Obtained data shows that better control of microbial lipopolysaccharide-endotoxin influence must be ensured in order to achieve sufficient results and prevent further systemic inflammation. This universal phenomenon may be valid not only for trauma itself but any liver injury including surgery, viral hepatitis and possibly autoimmune disease, depicting liver's importance in regulating immune regulation.

35. Genetically determined linkage of metabolism, hepatic functions, adipokines and CD4+ cells in MASLD

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Introduction: Metabolic dysfunction associated liver disease (MASLD) is a fatty infiltration of liver often leading to inflammatory damage to hepatocytes, closely related to metabolic syndrome, obesity, insulin resistance, type 2 diabetes mellitus, arterial hypertension, and hyperlipidaemia. Pathogenesis of MASLD is complex and combine metabolic, inflammatory, and immune components. Common mechanisms for hepatic steatosis include reduced synthesis of very low-density lipoprotein, and increased triglyceride synthesis; inflammation may result from lipid peroxidative damage to cell membranes, stimulating hepatic stellate cells, leading to liver failure and fibrosis. Number of experimental and clinical studies suggest that leptin (adipokine responsible for regulating body weight and energy expenditure) plays a key role to balance energy expenditure and nutritional status in the immune system. Moreover, leptin is also a crucial regulator of immunity and functions as a pro-inflammatory cytokine, influencing T-cell general population, particularly CD4+ cells proliferation, and balance between Th1 and Th2 phenotypes. Nonetheless, genetic background of orchestration apropos of leptin, hepatic function, metabolism and immune cells in MASLD is mostly unclear. Therefore, the aim of this study was to analyze the associations

of the ACE (I/D), PPAR-γ2 (Pro12Ala) genetic polymorphisms with hepatocytes' functional activity, leptin and immune cells in patients with MASLD.

Methods: This study fully conforms to international bioethical standards and involved 96 patients (mean age 53.70 ± 5.34 yrs) with MASLD, 56 (58.33%) women and 40 (41.67%) men. Diagnosis and management according to EASL-EASD-EASO Clinical Practice Guidelines. The liver function was assessed by detoxification, protein-synthetic function of hepatocytes indices, by the presence/absence of cytolysis, mesenchymal inflammatory syndrome, or cholestasis. Leptin and adiponectin were measured in ELISA. Leptin resistance (LR) was calculated as the leptin/triglycerides ratio. Quantification of CD4+ T-cells was performed by fluorescence microscopy after Acridine Orange staining following 3-step immunomagnetic cell separation in samples of EDTA anticoagulated blood. PCR was used to study the CNPs of PPAR-γ2 (Pro12Ala, rs1801282) and ACE (I/D, rs4646994) genes.

Results: Liver protein synthesizing function did not suffer, regardless of the genes' SNPs. In ACE DD genotype, direct bilirubin, AST/ALT, and CD4+ were higher than in II/ID-genotypes. Conjugated bilirubin was higher by 22.24% and 20.55% ($p < 0.05$), AST – 2.12 and 1.71 times ($p < 0.05$); ALT – 1.32 times ($p < 0.05$); CD4+ by 14.56% ($p < 0.05$). Ala-allele carriers had higher rates of AST, ALT as well as CD4+ compared to Pro12-genotype of the PPAR-γ2 gene – by 35.21%, 38.64%, 29.81% and 12.95%, respectively ($p < 0.05$). The fasting glucose level was higher in all patients regardless of type 2 diabetes, requiring further clarification. Leptin is higher in women, regardless of MASLD type and obesity by 1.74–2.39 times ($p < 0.001$). In MASLD men, leptin was 25.39% higher than in men with MASH, $p < 0.05$. In case of 3rd degree obesity, leptin was higher than in 1 and 2 degrees, regardless of gender: in men by 48.99% ($p = 0.022$) and 43.55% ($p = 0.034$); in women by 53.34% ($p < 0.001$) and 50.98% ($p = 0.002$). Obesity was associated with high leptin content in men ($F = 77.95$, $p < 0.001$) and women ($F = 341.43$, $p < 0.001$), with LR increase ($F = 103.17$, $p < 0.001$) and decrease of adiponectin ($F = 44.84$, $p < 0.001$). In female D-allele carriers of the ACE gene, leptin exceeded the one in II-genotype by 22.19% ($p = 0.048$) and 28.73% ($p = 0.036$). In Pro12 genotype of the PPAR-γ2 gene, leptin exceeded that of the Ala-allele carriers regardless of sex: in men 1.99 ($p = 0.036$) and 3.75 times ($p = 0.008$), in women by 32.79% ($p = 0.015$) and 27.81% ($p = 0.043$). LR prevailed exclusively in male homozygous Pro-allele carriers, over Ala-allele carriers – 2.23 and 3.16 times ($p < 0.01$), in contrast to women, where such dependences were not found, despite higher initial level of both leptins itself and LR rate.

Discussion/Conclusion: Inflammation is closely associated with the development of MASLD. Multiple factors including metabolic dysfunction, oxidative stress, and lipotoxicity have been confirmed to initiate inflammation in MASLD. Different studies demonstrated subpopulations of T-cells are involved in MASLD through interacting/supporting factors mentioned above. It is known that CD4+ cells can either protect the liver from infections or act a responsible actor in autoimmune hepatocellular injury. This study confirmed many previous findings and showed associations of adipocytokines activity, bilirubin, transaminases, and CD4+ in MASLD with genetic polymorphisms.

36. In vivo study of the natural killer T-cells in diet-related metabolic dysfunction associated liver disease

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Introduction: Metabolic dysfunction associated liver disease (MASLD) ranges from simple steatosis to metabolic dysfunction-associated steatohepatitis (MASH), progressing to cirrhosis

and hepatocellular carcinoma. Compared to MASLD, patients with MASH are more likely to develop advanced stages of liver fibrosis, cirrhosis and carcinoma. Although the pathogenetic mechanisms of disease progression remain uncertain, growing evidence suggests that the immune system participate in this process. Natural killer (NK) T-cells, an innate immune cells, are abundant in the liver, accounting for 20–35% of total hepatic lymphocytes in rodent experimental models and can specifically recognise glycolipid antigens, and produce both Th1 and Th2 cytokines when activated, and sharing characteristics with both T-cells and natural killer cells However, the role of NK T-cells in hepatic fibrogenesis is poorly understood. Moreover, while the role of diet in progression of MASLD and MASH is well established, the role of these immune cells remains unclear. Therefore, the aim of this study was to analyze the associations of diet with NK T-cells, proinflammatory cytokines and liver fibrosis in MASLD.

Methods: The study fully conforms to international bioethical standards and performed experimentally on rodent model. Fifty adult Wistar-line rats received 16 weeks of either Methionine-Choline-Deficient Diet (MCD, containing high sucrose and fat without methionine and choline, which are essential for hepatic mitochondrial β-oxidation and for synthesis of VLDL) diet, which is the classic dietary model for studying MASH, or the Fast-Food diet (FFD, containing high fat, high cholesterol, high fructose), which recently becomes another dietary tool for MASLD modelling. Fifteen animals formed control, receiving normal diet with not more than 10-12% of calories from fat. Liver histology, NK T-cells, fibrosis, and proinflammatory cytokines were studied in liver bioptates.

Results: Both diets significantly impacted body mass: FFD increased it by 95.36 ± 12.71% ($p < 0.01$), but MCD decreased it by 37.14 ± 11.09% ($p < 0.05$), supporting previous findings. Further changes in body mass were insignificant. FFD fed animals developed mainly perisinusoidal/pericellular histological changes associated with mild to moderate NASH 1 stage fibrosis, whereas MCD fed animals presented with paraacinar macrovesicular steatosis, severe inflammation, hepatocellular ballooning, more advanced stage of fibrosis (mostly 2–3 stages), occurring in a perisinusoidal/pericellular, perivenular or bridging fibrosis patterns, associated with severe MASH. FFD diet animals showed significant decrease of NK T-cells compared to both control and MCD group ($0.01 < p < 0.05$), while difference between control and MCD group was insignificant ($p = 0.11$). IL-4 remained unchanged in the FFD group ($p = 0.19$), while being significantly higher in MCD animals compared to control ($p < 0.01$).

Discussion/Conclusion: It is well known that inflammation and immune response play a key role in the pathogenesis of obesity-associated metabolic diseases, including MASLD/MASH. Studies on pathogenic and protective role of NK T-cells in MASLD are controversial: it was shown that lack of NK T-cells may promote steatosis, inflammation, and liver fibrosis in high-fat or choline-deficient diets, whereas other studies showed NK T-cells play a role in promoting liver fibrogenesis and cirrhosis. This study confirms existing associations of dietary habits and development of metabolism associated liver conditions.

37. Hepatic and extrahepatic complications in autoimmune hepatitis (case report)

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Introduction: Immune disorders in autoimmune hepatitis lead to both hepatic and extrahepatic manifestations.

Methods: A 20-year-old female was admitted with acute fulminant hepatitis. The patient's medical history was notable for bariatric surgery performed 1.5 years ago. Since then,

she had experienced intestinal dysfunction caused by dysbiosis. Acute viral hepatitis A, B, C, E, leptospirosis, toxic hepatitis were excluded, and the autoimmune hepatitis panel (ANA AMA SMA LKM-1 IgG) was negative according to laboratory tests. Detoxification and hepatoprotective therapy were initiated; however, due to the severity of the disease, steroids were added to the treatment regimen. Gradual improvement was observed. The clinical case was classified as idiopathic hepatitis. After discontinuation of treatment, the patient developed acne-like rashes that rapidly spread, becoming generalized. Dermatologists assessed the acne as a result of immunosuppression induced by steroid therapy, however traditional treatments for acne were ineffective.

Three months later, the patient experienced a recurrence of acute hepatitis. The condition worsened significantly. A liver biopsy was performed, and histological examination confirmed the diagnosis of autoimmune hepatitis. Treatment with prednisone and azathioprine was initiated, leading to stabilization of the condition, resolution of hepatitis symptoms, and improvement of the acne.

Results: In our case, acne was considered a side effect of post-steroid immunosuppression, and no suspicion was raised about autoimmune hepatitis. This delay in diagnosis and treatment initiation resulted in a relapse. The patient's stable remission was achieved only after prolonged immunosuppressive therapy.

Discussion/Conclusion:

1. Generalized acne that is resistant to standard treatment may be an extrahepatic manifestation of autoimmune liver damage.
2. In cases of hepatitis of unknown origin, laboratory markers alone may not be sufficient to rule out autoimmune hepatitis, and liver biopsy with morphological examination is important
3. Immunological disord.ers may lead to hepatic and extrahepatic manifestations and complication.

38. Inverse correlation between Th2-associated cytokines and hepatic egg burden in Schistosoma mansoni-infected hamsters

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Introduction: Schistosomiasis is a parasitic infection caused by Schistosoma species that afflicts over 250 million people worldwide. The Schistosoma mansoni species specifically targets the gastrointestinal system and triggers a Th2-type immune response through its eggs, resulting in granuloma formation. However, the relationship between the burden of parasitic eggs and the immune response remains inadequately understood. This study sought to examine whether the quantity of S. mansoni eggs influences the immune response in infected hamsters.

Methods: Eight-week-old hamsters were infected with S. mansoni cercariae using the paddling technique. Bisex and monosex worm populations were generated through poly-miracidial and mono-miracidial intermediate-host infections, respectively. The hepatic and intestinal egg burdens were quantified, and cytokine expression as well as the mRNA expression of three key egg-derived proteins (IPSE/alpha-1, kappa-5, and omega-1) were analyzed in monosex- and bisex-infected animals via qRT-PCR. Statistical analyses were conducted to determine the correlations between egg burden or egg-derived factors and the immune response in liver and colon tissue.

Results: Notably, the Th1 cytokine response induced by S. mansoni infection was independent of the hepatic egg burden, while the Th2 cytokines IL-4, IL-5, and IL-13 exhibited an inverse correlation in the liver. A modest cytokine expression was observed in the monosex-infected group, whereas bisex-infected animals showed up to 4.6-fold (IL-4), 10-fold (IL-5), and 30-fold (IL-13) increased expression levels. Furthermore, hepatic IL-4 and IL-13 expression correlated inversely with egg-derived factors of S. mansoni, such as IPSE/alpha-1, kappa-5, and omega-1. In contrast, the induction of IL-4, IL-5, and IL-13 mRNA expression in the colon tissue demonstrated no dependence on the intestinal egg burden.

Discussion/Conclusion: Our findings suggest an inverse correlation between hepatic egg burden, soluble egg factors, and the Th2 immune response in the liver of infected hamsters, but not in the colon. This implies an evolution of a protective mechanism by the host to restrain the Th2 response when confronted with a high hepatic egg burden. The protective mechanism is potentially due to the extended embryogenesis of the parasitic eggs in the liver. Our hypothesis is corroborated by the absence of any correlation between egg burden and immune response in the colon, where the intestinal transit of the eggs is more rapid.

39. Alcohol-induced fibrosis in a long-term 3D human liver model

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Introduction: Liver diseases, responsible for approximately 2 million deaths annually, often progress through fibrosis to liver failure, highlighting the importance of understanding the underlying mechanisms. Alcohol is a major factor in liver fibrosis and is associated with continuous activation of hepatic stellate cells (HSCs). While animal models are commonly used to study liver fibrosis, interspecies differences limit their translational relevance. Furthermore, short-term in vitro models fail to capture the complex progression of fibrosis adequately. Our study aims to develop a long-term human 3D in vitro model of alcohol-induced liver fibrosis.

Methods: Human liver progenitor cells (HepaRG), human umbilical cord endothelial cells (HUVEC), and HSCs (LX-2) were co-cultured to form liver spheroids, and exposed to 50 mM alcohol daily (exposure associated with intake of 14 grams of pure alcohol [standard drink]). To assess disease progression, we evaluated mitochondrial activity, total DNA levels, transcript genes levels related to HSCs activation, alcohol metabolism, and protein levels of transforming growth factor-beta 1 (TGF-β1), collagen precursor (PINP), and alkaline phosphatase (AP).

Results: Our model maintains stable functionality for at least 21 days. Mitochondrial activity and total DNA levels were not affected. However, alcohol exposure upregulated CYP2E1 gene expression and promoted HSCs activation, evidenced by increased fibroblast activation protein alpha transcript levels. These findings were further corroborated by bodipy staining and dot blot analysis, which showed a significant decrease in lipid droplets and an increase in TGF-β1, PINP, and AP protein levels, consistent with HSCs activation and fibrosis progression.

Discussion/Conclusion: Our 3D human liver model effectively simulates the long-term progression of alcohol-induced liver fibrosis. Under alcohol stimulation, hepatocytes secrete more TGF-β1, which continuously activates HSCs, causing the model to maintain a fibrotic state for a long time. Furthermore, our model provides a potential tool to investigate liver fibrosis-related diseases such as hepatic osteodystrophy.

40. T-cell receptor analysis of circulating T cells reveals non-epitope-driven clonal expansion in metabolic associated steatotic liver disease

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Introduction: Metabolic dysfunction-associated steatotic liver disease (MASLD) is characterized by hepatic steatosis, frequently resulting in inflammatory associated steatohepatitis (MASH), which in turn often leads to liver fibrosis or even cirrhosis. The immune system is crucial for these severe MASH alterations. In our study, we aimed to investigate the clonal expansion of circulating T cells during the progression of MASLD.

Methods: From a total of 210 obese patients who underwent bariatric surgery, liver biopsies were obtained intraoperatively and the MASLD progression was classified using the Kleiner score. Immune cells were studied by quantitative immunohistology. Buffy-coats were prepared from blood taken at the time-point of bariatric surgery. Simultaneous, multiplexed library preparation on 96 buffy-coats was performed using the one-step AccuraCode® technology, followed by ultra-deep sequencing. For T-cell receptor (TCR) analysis (identification of clonotypes, clones, gene usage, TCR-repertoires, diversity, motif, sequence studies) a bioinformatic pipeline was established.

Results: By targeting the TCR heterogeneity, clonal expansion was analyzed in 96 buffy-coats, selected according to patient´s MASLD progression: MASLD score 0–1 (n = 22), MASH score 2–4 (n = 41) and 5–7 (n = 33). Interestingly, there was no association of the number of circulating T-cell clonotypes with hepatic infiltration of various immune cell-types. However, we observed a significantly enhanced number of TCR-clonotypes along with development of severe MASH (MASH 5–7). Moreover, clonotype/clone ratios indicated a tendency towards a higher clonal expansion in patients with severe MASH. Most notably, TCR motif and sequence studies revealed that the expanded clonotypes were directed against epitopes of opportunistic or frequently common viral infections (CMC, EBV), but not to liver-specific antigens.

Discussion/Conclusion: These results provide primary evidence that a general, but not epitope-directed clonal T-cell expansion occurs during MASLD progression. Further insights into clonal T-cell activation will be gained by ongoing studies on expanded cohorts, healthy non-obese controls, and patients with MASH regression.

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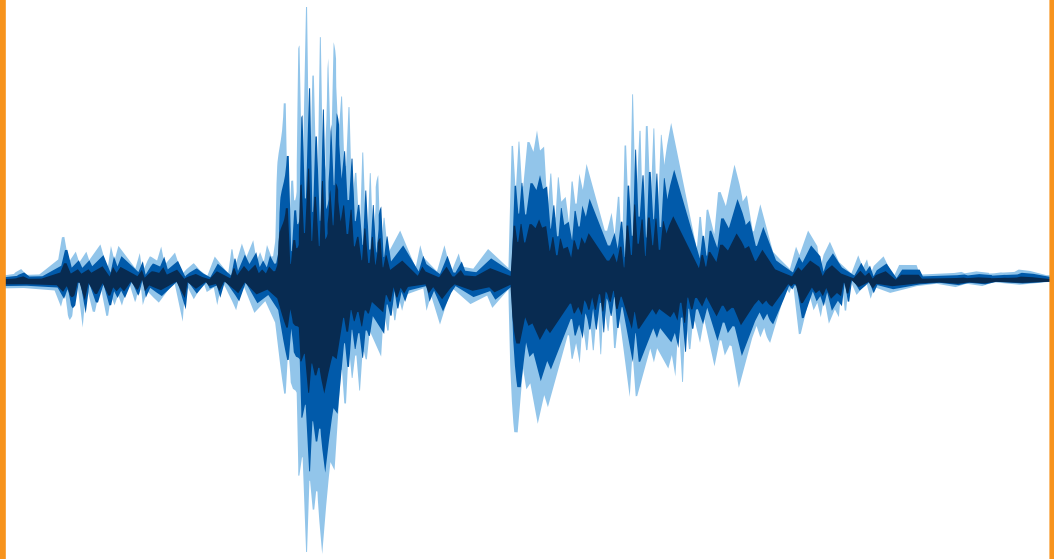

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